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Evaluation of SD BIOLINE rapid antibody test for diagnosis of *Helicobacter pylori* infection

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***Helicobacter pylori* infection is most prevalent and known to cause chronic gastritis and peptic ulcer disease in Ethiopia. To date, simple and rapid point-of-care tests are commercially available; however, information is limited regarding their diagnostic significance. This cross-sectional study was conducted to evaluate the diagnostic performance of SD BIOLINE *H. pylori* rapid antibody test. A consecutive 203 enzyme linked immunosorbent assay (ELISA) confirmed sera (148 *H. pylori* positive and 55 negative) from dyspeptic patients were tested using SD BIOLINE *H. pylori* kit (Standard Diagnostic Inc, Korea) at Hawassa Teaching and Referral Hospital, southern Ethiopia from October, 2012 to January, 2013. Individuals under 15 years old, who were on anti- *H. pylori* treatment during the month prior to the study, those with discordant ELISA results, and refused to participate, were excluded. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the SD BIOLINE *H. pylori* test kit were 95.3, 94.5, 97.9, 88.1 and 95.1%, respectively. Therefore, this point-of-care test could be used as alternative to ELISA testing and best fit our context.**

Key words: *Helicobacter pylori*, point-of-care, enzyme linked immunosorbent assay (ELISA), SD BIOLINE.

INTRODUCTION

Helicobacter pylorus is a small, spiral, gram-negative bacillus that appears to inhabit the mucous layer overlying the gastric epithelial cells in humans. It was formerly known as *Campylobacter pyloridis* then *Campylobacter pylori* (Engstrand and Lindberg, 2013). An estimated 50% of the world's population is infected; yet, its principal mode of transmission remains largely unknown. It might be due to poverty related factors that the prevalence of *H. pylori* infection is markedly higher in developing countries than in developed nations (Khalifa et al., 2010). In Ethiopia (Addis Ababa), an investigation using different diagnostic methods showed *H. pylori* prevalence varying between 69 and 91% among adult dyspeptic patients (Asrat et al., 2004). Similar high prevalence of *H. pylori* infection (70%) was also shown in dyspeptic patients in northwest Ethiopia (Bahir-Dar) (Tadege et al., 2005). The bacterium is known as the most common cause of chronic gastritis and peptic ulcer disease. Also, infection is associated with gastric adeno-carcinoma, which is the deadliest cancer (McColl and El-Omar, 2002). It has been shown that eradicating *H. pylori* infection reduces ulcer relapse (Arkkila et al., 2005) and the risk of gastric cancer development among patients with or without precancerous lesion (Wong et al., 2004, 2012).

H. pylori can be diagnosed using invasive and non-invasive methods; but a combination of at least two methods has been recommended to improve accuracy of

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its detection (Ramis et al., 2012). The invasive methods such as culture, histology and rapid urease testing of endoscopic biopsy from gastric mucosa are commonly used. But these methods are tedious and time consuming and require sampling procedure that cause discomfort to patients (Cirak et al., 2007). The non-invasive methods include ^{13}C and ^{14}C urea breath tests (UBTs) and serology. UBTs are specific for active infection; however, they are more expensive or have safety concern, limiting their diagnostic suitability (Mansour-Ghanaei et al., 2011; Kato et al., 2002). In contrast, serological tests are inexpensive and widely available even though enzyme linked immunosorbent assay (ELISA) based tests require costly laboratory equipment, trained personnel and electric supply to establish diagnosis in resource-constrained settings (Leal et al., 2007).

To date, rapid diagnostic tests (RDTs) detecting antibodies against *H. pylori* infection are commercially available and widely applied in clinical practice in our settings. RDTs are simple to perform, has short test time and do not require laboratory support. However, studies reported that RDTs show varying level of diagnostic performance, which is influenced by *H. pylori* prevalence, genetic diversity and study population (Chen et al., 1997; Elitsur et al., 1997; Pelerito et al., 2006). Thus, it highlights the need to assess the diagnostic usefulness of RDTs in our context where *H. pylori* is prevalent; but little is known regarding RDT performance characteristics. This study was conducted to evaluate the diagnostic performance of SD BIOLINE *H. pylori* rapid antibody test so that its diagnostic value to serve as alternative to ELISA would be elucidated.

MATERIALS AND METHODS

Study subjects

A cross-sectional study was conducted in consecutive dyspeptic patients who provide blood for *H. pylori* serological testing at Hawassa Teaching and Referral Hospital, southern Ethiopia from October, 2012 to January, 2013. Two hundred and three ELISA confirmed sera (148 *H. pylori* positive and 55 negative) were used. There were 11 patients that were excluded: 3 were on anti-*H. pylori* treatment during the month prior to the study; 1 refused to participate; 4 had discordant ELISA results; 3 were children under 15 years old. The study obtained ethical clearance from the Institutional Review Board of College of Medicine and Health Sciences, Hawassa University.

Serology

Venous blood was collected from the study participants and sera were stored at -20°C until analyzed. All sera were tested for anti-*H. pylori* immunoglobulin G (IgG) antibody using two different ELISA kits: Pyloriset EIA-G III (Orion Diagnostica, Germany) and *H. pylori* IgG ELISA (IBL International, Hamburg, Germany). According to the manufacturers' claim, the respective sensitivity and specificity were 100 and 94.3% for Pyloriset EIA-G III and > 95 for *H. pylori* IgG ELISA. In this study, *H. pylori* infection status was determined on the bases of the same positive or negative results obtained by both ELISA tests.

ELISAs

Pyloriset EIA-G III testing

A 100 µl of each calibrator sera (labeled 1 to 4) and 100 µl of each 1 to 201 diluted patient sera were pipetted to microtiter wells coated with the specific *H. pylori* antigen. Plates were incubated on a plate shaker (1000 rpm) at 25°C for 30 min. After washing 3 times, 100 µl of enzyme conjugate was added into the wells and incubated again at 25°C for 30 min. Following washing step, 100 µl of substrate solution was added and plates were incubated at 25°C for 10 min. The reaction was stopped by adding 100 µl of 0.5 M sulfuric acid and the optical density (OD) of each well was read at 450 nm within 10 min after stopping. As specified by the manufacturer, samples were defined as positive when their OD values are equal to or higher than that of the calibrator serum 2 OD.

H. pylori IgG ELISA testing

A 100 µl of each standard (labeled A to D) and 100 µl of each 1 to 101 diluted patient sera were added into the respective microtiter wells. Plates were covered with adhesive foil and incubated at 25°C for 60 min. After washing 3 times with washing buffer, 100 µl of enzyme conjugate was added into each well and incubated at 25°C for 30 min. Following similar washing steps, 100 µl of TMB substrate solution was added into each well and incubated at 25°C for 20 min. The reaction was stopped by adding 0.5 M sulfuric acid, and the OD of each well was read at 450/630 nm within 60 minutes after stopping. As per the instruction of the manufacturer, the OD of each sample was divided by the cut-off standard (standard B) value to obtain a cut-off index (COI). A respective COI value of < 0.8, 0.8 to 1.2 and > 1.2 were interpreted as negative, equivocal and positive.

Rapid diagnostic test

Those ELISA characterized sera were tested using a commercial rapid diagnostic kit, SD BIOLINE *H. pylori* kit (Standard Diagnostic Inc, Korea). A single investigator blinded to the results of ELISA read all the rapid test results. Tests were carried out according to instruction of the manufacturer. The SD BIOLINE *H. pylori* antibody test is a simple one step immune-chromatographic assay for qualitative detection of antibodies of all isotopes to *H. pylori.* The test procedure is to add 10 µl of serum, plasma or whole blood to a sample well, and then adding 3 drops of assay diluent to the same well. The result was read at 10 min. Positive test result is indicated when control (C) and test line (T) are visible, and negative result is when only control line (C) is visible. The test is invalid when the control line is invisible. Data entry and analysis was performed using STATA Version-10. Results are summarized using mean and proportion as appropriate. Parameters including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated considering concordant ELISA results as true results.

RESULTS

Sera from 203 patients (102 male, 101 female) were characterized for the *H. pylori* status by ELISA testing. They

Table 1. Serology results of SD BIOLINE *H. pylori* antibody test kit, southern Ethiopia, 2012-2013.

RDT	ELISA Testing		Total
	Positive	Negative	
SD BIOLINE *H. pylori*			
Positive	141	3	144
Negative	7	52	59
Total	148	55	203

RDT, rapid diagnostic test; ELISA, enzyme-linked immunosorbent assay

Table 2. The diagnostic performance characteristics of SD BIOLINE *H. pylori* antibody test kit, southern Ethiopia, 2012-2013.

RDT	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
SD BIOLINE *H. pylori* kit	95.3 (90.5-98.1)	94.5 (84.9-98.9)	97.9 (94-99.6)	88.1 (77.1-95.1)

RDT, rapid diagnostic test; PPV, positive predictive value; NPV, negative predictive value.

were aged between 15 to 78 years, with a mean age of 36.9 years and standard deviation 14.8 years. One hundred and forty eight (72.9%) sera were positive for *H. pylori* IgG antibody and 55 (27.1%) were negative. There was an agreement between the SD BIOLINE *H. pylori* and ELISA results in 193 of 203 sera (overall accuracy 95.1%). As presented in Table 1, 141 true-positive, 52 true-negative, 3 false-positive and 7 false-negative results was obtained by SD BIOLINE *H. pylori* test kit. Out of 144 positive results, 8 were faint positive (Figure 1). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of SD BIOLINE *H. pylori* rapid test were 95.3, 94.5, 97.9 and 88.1%, respectively (Table 2).

DISCUSSION

In our context where lack of resources and expertise limit regular evaluations of diagnostic methods, many new tests are marketed and used directly without prior evaluation for their appropriateness. Rapid serological kits for *H. pylori* are among the most widely used tests as these products are easily applicable and other diagnostic methods are limited. However, the need to determine their diagnostic value through evaluation should be emphasized for the improved management and control of *H. pylori* infection. In attempt to find a reliable point-of-care test, we evaluated the diagnostic performance of SD BIOLINE *H. pylori* rapid antibody test. The sensitivity, specificity, PPV and NPV of the test kit were 95.3, 94.5, 97.9 and 88.1%, respectively. The kit's manufacturer claimed similar performance characteristics: sensitivity (95.9%), specificity (89.6%), PPV (93.4%), and NPV (93.5%). Also, the overall accuracy (95.1%) we reported was in accordance with that of the manufacturer (93.4%).

The various diagnostic products, dissimilar methodologies and diverse studied population may limit direct comparison between findings coming out of validation studies. In agreement with our findings, a previous evaluation of the Assure *H. pylori* rapid serological test (Genelabs Diagnostics, Singapore) reported a respective sensitivity and specificity of 90.1 and 80.9% in Bangladeshi adult patients (Pelerito et al., 2006). Moreover, evaluation of the Assure *H. pylori* in Portuguese pediatric population showed compatible specificity (95%) to that of the SD BIOLINE *H. pylori* kit, although its sensitivity (75.7%) was inferior (Rahman et al., 2008). However, unlike our evaluation, those studies included methods such as culture, rapid urease test, and histology to confirm patients' *H. pylori* status.

The NPV of SD BIOLINE *H. pylori* kit suggests that negative results could be used to rule out *H. pylori* infection. Also, the observed high PPV of the test kit indicates the reliability of positive results to detect *H. pylori* exposure, truly. Nonetheless, the faint positive results may increase inter-observer variation and the risk of reporting false-negative result. However, since the predictive values of a test depend on the prevalence of *H. pylori* infection (Banoo et al., 2010), the test kit may demonstrate different performance elsewhere if the prevalence is lower.

Serology is a non-invasive method detecting antibody against *H. pylori* infection and it is the only test unaffected by changes in the stomach that could lead to a low bacterial load and to false-negative results of the other

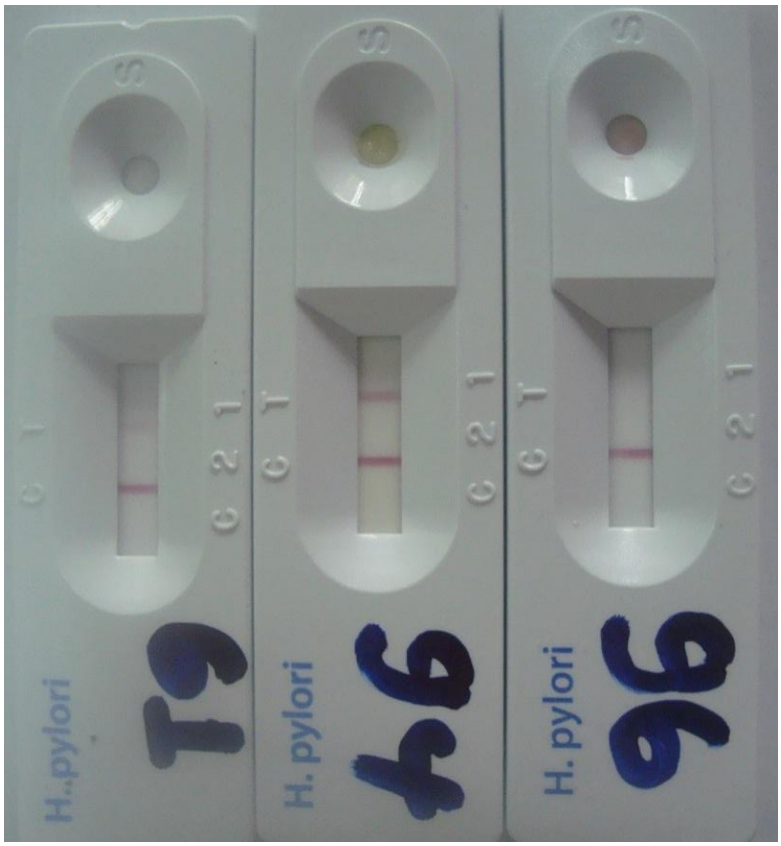

Figure 1. Photograph of SD BIOLINE *H. pylori* rapid antibody test results.
Left: faint positive; Middle: Positive; Right: Negative (61, 94, and 96: Patients' identification numbers).

tests (Malfertheiner et al., 2012). However, serology is unable to distinguish between active infection and past exposure due to the fact that antibodies against *H. pylori* remain elevated for longer period after the disappearance of the infection (Ekstrom et al., 2001). In Ethiopian setting with a high prevalence of *H. pylori* infection (Asrat et al., 2004; Tadege et al., 2005), ELISA was shown to be highly accurate in diagnosing dyspeptic patients (Asrat et al., 2007). However, ELISA-based *H. pylori* serological test takes longer time, requires trained staff and a laboratory set-up; thus, it has application difficulties in resource-limited developing countries (Leal et al., 2008). The SD BIOLINE *H. pylori* rapid antibody test addresses challenges related to ELISA as it is simple to test, has short test time (less than 30 min), does not require laboratory equipment or electricity supply, and use whole blood, plasma or serum as diagnostic sample.

However, the results of this study should be interpreted in light of its methodological limitation as the tests employed for *H. pylori* diagnosis did not include methods of choice such as culture, UBTs and histology against those comparisons of the serological tests would have been imperative.

Conclusion

The SD BIOLINE *H. pylori* rapid antibody test was found sensitive and specific for screening of *H. pylori* infection in Ethiopian populations. Therefore, this point-of-care test best fit our context and could be used as alternative to ELISA testing.

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