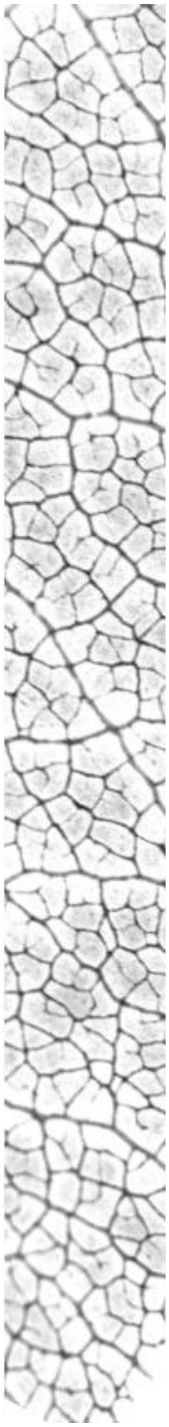

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Diagnostic value of *Helicobacter pylori* serum serology using immunochromatography method with current infection marker compared to histopathology in dyspeptic patients

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Abstract

Introduction: Examination using Outer Membrane Protein as a marker of active *Helicobacter pylori* Current Infection Marker (CIM) infection has now been developed. However, the accuracy of CIM is still unknown. This study aimed to analyze the diagnostic value of serum serology using CIM compared to histopathological examination as the gold standard for diagnosing the presence of *H. pylori* infection in dyspepsia patients.
Methods: This study involved fifty-two subjects with dyspepsia. Endoscopic, biopsy, and histopathological examination with modified-giemsa staining as gold standard and serological examination using immunochromatography method with CIM (Assure^{R}, Singapore) had been done to all subjects of this study.
Results: Pre-test probability of *H. pylori* infection was 17.3%. Most of subjects infected with *H. pylori* were male with average age of 53.89±7.75 years old. Most of endoscopic features of subjects infected with *H. pylori* were erosive gastritis. The sensitivity, specificity, positive predictive value, negative predictive value of CIM were 22.2%, 95.3%, 50%, and 85.4%, respectively. The positive likelihood ratio, negative likelihood ratio, and accuracy of CIM were 4.8, 0.8, and 82.7%, respectively.
Conclusion: Serum serology using immunochromatography with CIM cannot replace histopathology for diagnosis of *Helicobacter pylori* current infection in dyspeptic patients.

Keywords: *Helicobacter pylori*, immunochromatography, current infection marker, dyspepsia

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INTRODUCTION

Dyspepsia is a pain symptom aggregate or feelings of discomfort in the area of the upper gastrointestinal tract that is ongoing chronically and recurrent (Moayyedi et al. 2017). Analytical studies proved that *Helicobacter pylori* has a major role in gastrointestinal diseases that manifest as symptom of dyspepsia (Matsuda et al. 2009). *H. pylori* infection has been classified as a class 1 carcinogen in 1994 by the International Agency for Research on Cancer (World Health Organization) and become a major risk factor associated with gastric cancer by 31% -92% (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 1994, Siregar et al. 2015). *H. pylori* strains have so many varieties, and Indonesia is dominated by high virulence

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strains, namely *East Asian CagA, VacA S1-m1,* and *OipA'On* 'although Indonesia is classified as a country with a low risk of gastric disease (Miftahussurur et al. 2015, Siregar et al. 2018). Besides, Indonesian strains have the high prevalence of some antibiotics resistance (Miftahussurur et al. 2016).

Establishment of the *H. pylori* infection diagnosis in Dr. Soetomo General Hospital in Surabaya still becomes a problem because it still requires an invasive method, takes longer to wait for the histopathology results, and depends on the location of colonization of germs. Non-invasive methods such as Urea Breath Test (UBT) and Stool Antigen Test (SAT) have not been used, whereas serology test accuracy by using a new method called *Current Infection Marker* (CIM) on *H. pylori* strains in Indonesia specifically at Dr. Soetomo General Hospital Surabaya has not been known yet (Asha Jose et al., 2019).

The prevalence of *H. pylori* has many varieties from 20%-50% in developed countries, whereas in developing countries it may reach 90% (Jemilohun et al. 2016). The varieties data on the *H. pylori* infection prevalence in Indonesia are between 2-68% with different diagnostic methods (Abdullah et al. 2009, SyamAri Fahrial et al. 2005, Tokudome et al. 2005). Colonization of *H. pylori* can increase the risk for active chronic gastritis that can later develop into peptic ulcer and malignancies, such as gastric cancer (Kusters et al. 2006). Ulcer-related deaths due to *H. pylori* are as many as 6500 deaths each year in the United States, although the prevalence of *H. pylori* infection is low (Fleming 2007). Gastric cancer is the fifth most common cancer and the third leading cause of cancer deaths in the world with 74,000 deaths per year (Oleastro et al. 2013, World Health Organization 2018).

The Bangkok Consensus Report (2018) recommended one of the *H. pylori* gold standard examinations in ASEAN, including Histology, Rapid Urease Test (RUT), and UBT. The choice of examination depends on the availability of equipment, price, and choice of patients (Mahachai et al. 2018, SyamA F et al. 2015). The gold standard of examination that has been used at Dr. Soetomo General Hospital in Surabaya is a histopathological examination because of its availability and costs. This examination has a diagnostic value of >95% but it is invasive, requires a long time to wait for the reading result, the result depends on the histopathologic and is influenced by biopsy location taking on the corpus and antrum. In addition, some studies reported that the staining methods are commonly used to identify the bacteria more clearly and quite simple, and the reagent is easy to obtain and cheap (Sandhika 2019: Jafarzadeh et al, 2018).

The non-invasive methods which are recommended for *H. pylori* infection: UBT, SAT, and serology. Urea Breath Test has a diagnostic value of ≥95% but it requires special tools, the examination costs are also more expensive, and the diagnostic value is influenced by the use of Proton Pump Inhibitors (PPI), bismuth, previous antibiotics, and the presence of other urease-producing germs. Stool Antigen Test examination has a diagnostic value of >90% but it is affected by the consistency of feces, and collection cannot be done at any time. Conventional serological test which uses the Enzyme-Linked Immunosorbent Assay (ELISA) is cheaper, simpler, does not require special expertise, and shows high conformity, but has lower specificity because it is unable to distinguish current and past infections (Graham et al. 2001, Hutagalung et al. 2009).

Examination using Outer Membrane Protein as a marker of active *H. pylori* Current Infection Marker (CIM) infection has now been developed (Suerbaum et al. 2002). The CIM protein is an antigen that has homologous nucleotide sequencing with the outer membrane of the *H. pylori* protein. The CIM protein has a high immunogenic detection in patients actively infected by *H. pylori* with positive predictive value of >90%. Previous study compared between Immunochromatography (ICT) and Immunoblot examinations used CIM with conventional serological examinations using ELISA obtained higher specificity on ICT and immunoblot using CIM of 90.4% and 96.3% compared to ELISA of 42.8% (Rahman et al. 2008). Non-invasive examination methods using ICT with CIM are expected to help establishing the diagnosis of current *H. pylori* infection quickly and accurately so that *H. pylori* infection treatment can be given immediately. This study was conducted to analyze the diagnostic value of serum serology by immunochromatography using CIM compared to histopathological examination as the gold standard for diagnosing the presence of *H. pylori* infection in dyspepsia patients.

METHODS

Study Design

This study was a cross-sectional test with a population of all dyspeptic patients who came to the outpatient unit-endoscopy of Gastroenterology-Hepatology Division of Internal Medicine Department, Dr. Soetomo General Hospital, Surabaya.

Selection of the Patients

The sampling method was done by consecutive sampling. Inclusion criteria were a minimum age of 18 years to 70 years, outpatients Gastroenterology Clinic of Dr. Soetomo General Hospital, Surabaya, with complaints of dyspepsia least in the last 3 months, dyspepsia patients with indications of upper gastrointestinal endoscopy including dyspepsia patients who did not improve by giving empirical therapy for 2-4 weeks, patients diagnosed with dyspepsia early at age ≥ 50 years, dyspepsia sufferers with alarm sign (unintentional decrease in weight, iron deficiency

anemia, dysphagia, odynophagia, persistent vomiting, first-degree family history of upper gastrointestinal malignancy, abnormal features from radiological examinations leading to organic abnormalities assessed by consultants of Gastroenterology-Hepatology), and was willing to take part in this research by signing an informed consent for an upper gastrointestinal endoscopy, gastric biopsy and venous blood sampling to be examined for serum serology after endoscopy. Whereas, the exclusion criteria were patients who experienced upper and lower gastrointestinal bleeding clinically, there were contra indications both absolute and relative for endoscopic examination and gastric biopsy assessed by a consultant of Gastroenterology-Hepatology included patients who refused to do endoscopic examination, uncooperative, had uncontrolled coagulopathy disorders, had heart disease unstable or acute myocardial infarction, respiratory failure and patients who were pregnant, patients with a history of gastric surgery, and patients with chronic kidney disease undergoing dialysis. This study has obtained ethical feasibility from the ethics committee in Health Research of Dr. Soetomo General Hospital, Surabaya, and informed consent was obtained from each subject of this study.

Determining of H. Pylori Infection

Histological Examination

Histopathological examination of *H. pylori* bacteria in the gastric mucosa is a gold standard procedure for knowing *H. pylori* germs microscopically by two anatomical pathologists, using a gastric mucosal biopsy material derived from the corpus and antrum which is processed and using a special stained modified Giemsa (Graham et al. 2001). The instruments used in this study were microscopic and specifically Giemsa (diff-quick) staining. The data were nominal scale data (*H. pylori* infection and no *H. pylori* infection). *H. pylori*'s appearance is like a curve or bacillus in the form of a spiral that is on the surface of the epithelium or in the mucosal layer and in a blue gastric pit against a light blue mucus background that is read by two anatomic pathologists and calculated for Kappa values (Lee et al. 2015).

The biopsy material was processed according to the standard procedure of making histopathological preparations into paraffin blocks. Paraffin blocks were sliced 3-4 microns thick with American Optical brand macrotomes, each made 2 pieces. For tissue histopathology examination, 1 slide was made for each of 4 and colored with modified giemsa (diff-quick) staining. The preparation was examined by 2 anatomic pathologists by looking at the presence or absence of *H. pylori* infection. Anatomical pathologist in conducting the examination did not know the results of endoscopy, nor the serological examination of serum. After that, Kappa values were calculated to determine interobserver variability.

Serology Immunochromatography with CIM Examination

Serological examination of the immunochromatography method with CIM is a qualitative indirect examination by measuring serum immunoglobulin G in patients infected by *H. pylori* arising from the presence of CIM protein antigens in the examination kit membrane. The method used in this examination was immunochromatography. This study used the tool called by Assure^{R} (MP Diagnostic, Singapore). Serological serological examination was done by immunochromatography method with CIM using a tool from Assure^{R} which was done by taking a blood sample just before endoscopic examination. Fasting blood was drawn from all subjects as much as 1 ml in EDTA tubes then before 2 hours in centrifuge for 15 minutes at 1500 rpm and stored at -20 C until it was used (less than 7 days). The serum was then dropped into the examination kit and examined according to the procedure in the examination kit brochure. Results were read after 15 minutes to a maximum of 20 minutes by trained clinical pathologists. If the control line (A), the CIM line (B) and the test line (C) were pink, it could be said to be positively infected by active *H. Pylori*. If a control line (A) was obtained, and the test line (B) was pink, it was said to be positive for *H. Pylori*, but it was not active at this time. The test results were said to be negative if only the control line (A) was only pink, whereas it was said to be invalid if only the pink was found on the CIM line (B) and the test line (C) or the test line (C), or the control line (A) and the CIM (B) line only (19). In this study, it was said to be positive for *H. pylori* infection based on serology examination by immunochromatography with CIM if the results of the examination showed positive for current *H. pylori* infection (control line (A), CIM line (B) and pink test line (C)).

Statistical Analysis

The collected data were processed by textual and tabular data. The results of the study were presented in the form of a 2x2 table to calculate diagnostic values in the form of sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio, and accuracy. An interobserver analysis was performed on the histopathological results with two readers so as to obtain kappa scores. In this study, the Open EPI software program was used.

RESULTS

Characteristic of Subjects

This research was conducted from February 2019 to May 2019. There were fifty-two patients who met the inclusion and exclusion criteria. The characteristics of subjects are presented in **Table 1**. A total of 9 patients

Table 1. Characteristics of subjects

Characteristics	Total Subjects n(%)	Infected with *H. pylori* by Histopathology n(%)	Not Infected with *H. pylori* by Histopathology n(%)
Total Subjects	52 (100)	9 (17.3)	43 (82.7)
Sex n (%)			
Male	23 (44.2)	5 (55.6)	18 (41.9)
Female	29 (55.8)	4 (44.4)	25 (58.1)
Age (Years)			
Mean±SD	49.5±9.9	53.9±7.8	48.5±10.2
Ethnicity - n (%)			
Javanese	35 (67.3)	6 (66.7)	29 (67.4)
Tionghoa	10 (19.2)	3 (33.3)	7 (16.3)
Ambonese	2 (3.8)	0 (0)	2 (4.7)
Maduranese	2 (3.8)	0 (0)	2 (4.7)
Dayaknese	1 (1.9)	0 (0)	1 (2.3)
Balinese	1 (1.9)	0 (0)	1 (2.3)
Bataknese	1 (1.9)	0 (0)	1 (2.3)
Symptoms - n (%)			
Epigastric pain	48 (92.3)	8 (88.9)	40 (93)
Nausea	40 (76.9)	5 (55.6)	35 (81.4)
Early satiation	34 (65.4)	7 (77.8)	27 (62.8)
Bloating	36 (69.2)	7 (77.8)	29 (67.4)
Heartburn	24 (46.2)	2 (22.2)	22 (51.2)
Vomitting	7 (13.5)	0 (0)	7 (16.3)
Weight loss	25 (48.1)	5 (55.6)	20 (46.5)

Table 2. Endoscopic Features of the subjects

Endoscopic Features	Infected with *H. pylori* by Histopathology (n=9)	Not Infected with *H. pylori* by Histopathology (n= 43)
Superficial gastritis	2 (22.2)	29 (67.4)
Erosive gastritis	4 (44.4)	11 (25.6)
Ulcus pepticum	3 (33.3)	1 (2.3)
Mass	0 (0)	0 (0)
Gastric atrophy	0 (0)	1 (2.3)
Normal finding	0 (0)	1 (2.3)

Table 3. Histological Examination Results which was Read by Two Different Pathologists

		Pathologist 2		Total
		Positive	Negative	
Pathologist 1	Positive	9	0	9
	Negative	0	43	43
Total		9	43	52

out of a total of 52 positive patients were infected by *H. pylori* with a gold standard examination, namely histopathology, so the probability of pre-testing of *H. pylori* infection in this study was 17.3%. Subjects infected by *H. pylori* had a higher proportion of men (55.6%) than women (44.4%) with an average age of 53.9 ± 7.8 years. Whereas, the subjects who were not infected were mostly female (58.1%), with an average age of 48.5 ± 10.2 years. A total of 6 out of 9 (66.7%) research subjects were infected by *H. pylori* came from the Javanese. The results of this study, together with a group of subjects who were not infected by *H. Pylori*, were found to be mostly Javanese (67.4%). However, the frequency of *H. pylori* infection was more prevalent in Chinese (3 out of 10 subjects, 30%) compared to Javanese (6 out of 35, i.e., 17.1%). The most common complaint from dyspepsia patients in both *H. Pylori*-infected and uninfected subjects was heartburn (88.9% and 93%, respectively).

The endoscopic features in this study were grouped according to the types of visual lesions and the involvement of gastric and duodenal heaviest. In subjects infected by *H. Pylori*, most of the endoscopic features found was erosival gastritis (44.4%), whereas in the majority of uninfected patients (67.4%), superficial gastritis was found. Peptic ulcers were more common in the *H. Pylori*-infected group (33.3%) than in the uninfected group (2.3%). Gastric atrophy was 2.3% in the *H. Pylori*-uninfected group. There were no endoscopic features in the form of mass in this study (**Table 2**).

The criteria in this study stated that patients were tested positive for *H. pylori* at this time if *H. pylori* was found in both histological examination results as a gold standard with a special staining of modified Giemsa (Diff-quick) which was read by two different anatomical pathologists (**Table 3**). The results of the examination conducted by the first reader got 9 subjects who were infected by *H. Pylori*, as well as the second reader got 9 subjects who were positive for *H. Pylori*. Both the first reader and the second reader got 43 subjects who were not infected by *H. Pylori*. Kappa interobserver values were calculated to obtain a result of 1 with a significant P = 0.00, which means that the observations between expert 1 and expert 2 had a very good level of conformity because of the Kappa 1 value.

Diagnostic Value of Serum Serology Using Immunochromatography Method with Current Infection Marker

As many as fifty-two patients participated in this study.In histopathological examination, 9 patients (17.3%) was found to be infected by *H. Pylori*. *H. pylori* examination of immunochromatography using CIM found 4 patients (7.7%) who showed positive results of being infected by *H. pylori* at this time. Two patients were positive, both with histopathological examination and serology, while 41 patients showed negative results, both with histopathological and serological examination. From 4 patients who showed positive results by serology method, there were 2 patients (50%) with

Table 4. Table 2x2 Serum Serology Compared with Histopathology

		Histopathology		Total
		Positive	Negative	
Serum Serology	Positive	2	2	4
	Negative	7	41	48
Total		9	43	52

Table 5. Diagnostic Value of Serum Serology using Immunochromatography Method with Current Infection Marker

	Diagnostic Value	95% Confidence interval
Sensitivity	22.2%	6.3%-54.7%
Specificity	95.3%	84.5%-98.7%
False Positive	4.7%	
False Negative	77.8%	

histopathological results that showed positive results. A total of 48 patients showed negative results by serology, 41 of them (85.4%) showed histopathological results which were also negative (**Table 4**).

The diagnostic test results of *H. pylori* infection with serum serology examination immunochromatography method with CIM compared to histopathology using modified Giemsa (Diff-quick) staining as gold standard obtained sensitivity and specificity of serum serology by 22.2% and 95.3%, respectively. Estimated value positive (NDP)/positive predictive value (PPV) serological examination of *H. pylori* serology by immunochromatography method in this study was 50%. Whereas, the negative predictive value (NPV) in this study was higher at 85.4%, the RKP was 4.8 (0.1-39.0), and the RKN value was 0.8 (0, 6-1,1). Overall, the accuracy obtained from serum serology examinations in this study was 82.7% (**Table 5**).

DISCUSSION

Overall serum serology examination using immunochromatography method with CIM cannot be used to rulling in or rulling out the diagnosis of *H. pylori* infection. The difference in strains of *H. pylori* can express different antigens so that the antibodies formed cannot be captured by this serum serology tool.

The results of this study differred from a previous study in China that assessed the accuracy of serum serology examination with immunochromatography using CIM. Their study using histopathological gold standard and [^{13}C] UBT showed quite high sensitivity and specificity in serum serology examination of 93.2% and 90.5%, as well as other studies using histopathological gold standard, Rapid Urease Test, and [^{13}C] UBT which showed the sensitivity and specificity of serological examination with ICT CIM which were quite high at 94% and 90% (Hung et al. 2002, Wang et al. 2008). Another study in Bangladesh in 2008 used the gold standard of histopathology, culture and Rapid Urease Test which found the sensitivity and specificity of serological examinations with ICT CIM which were high at 88.5% and 90.4% (Rahman et al. 2008).

In the current study, there were several possible causes for the low sensitivity and high false negative rates serological examination when compared to the three previous studies. Research on *H. pylori* gene polymorphisms has been widely studied, especially those related to the virulence of germs. It was known in China that the most strain was CagA East-Asian type with 39bp deletion. Likewise, the serum serology tools used in this study were produced by Singapore where the H strain, the most pylori (98.8%), was the CagA East-Asian ABD type (Hua et al. 1998, Leung et al. 1998, Lui et al. 2010). Research on *H. pylori* strains in Indonesia reported that there are differences in *H. pylori* strains in Indonesia with Western and East-Asian types, namely CagA East-Asian type with 6bp deletion, and most in Java is CagA East-Asian ABB type (Miftahussurur et al. 2015). The second cause of the low sensitivity and high enough false negatives is the failure of the device to detect antibodies in serum which can occur because the subject does not produce specific antibodies with an adequate amount of CIM antigens used in serum serology devices. Other causes are possible because of the influence of reading the results of serum serology tests. In accordance with the instructions obtained in the inspection kit, the reading of the results is done after 15 minutes, and it is not recommended to re-read more than 20 minutes. However, a study in Portugal found that the sensitivity and specificity values of serum serology examination with immunochromatography method will increase after reading at 45 minutes (After 15 minutes, Sn 75.7%, Sp 95%, whereas after 45 minutes, Sn 98.6% and Sp 95%) (Pelerito et al. 2006). The reason of increased reading time was due to the existence of dubious results, so researchers increased reading time. After 45 minutes, the chromatography zone was completely dry and the color indicators on the band became more visible (Pelerito et al. 2006).

The results of this study found a high specificity value and a relatively small false positive. The first cause is due to the presence of CIM so that this serological tool more specifically distinguishes current infection from past infection (Wang et al. 2008). The second cause is thought to be due to *H. pylori* in the form of cocoid. Where in unfavorable conditions, *H. pylori* bacteria will change shape to cocoid but still infectious, so antibodies can still be formed which can be detected by serological serums, but it is difficult to identify using histopathological examination with modified-giemsa staining. This is because the cocoid formation is not easily detected in histopathological examination and requires a special staining, immunohistochemistry (Wang et al. 2008).

The positive predictive value and negative predictive value of serum serology examination using immunochromatography method in this study were different from previous studies which showed that

likelihood ratio positive (LR+) and likelihood ratio negative (LR-) were both quite high. A study in China using the gold standard of culture, histopathology, and rapid urease test proved that serum serology examination using immunochromatography method with CIM had a LR+ of 82.7% and LR- of 88.9% (Peng et al. 2009). Likewise, a study in Bangladesh showed a high LR+ value of 96.4% and LR- 73.0% (Rahman et al. 2008). The cause of this difference was related to the high prevalence of *H. pylori* infection in both countries (>50%) and both studies using more than one gold standard, namely culture, histology and rapid urease test (RUT) so that readings were more accurate (Peng et al. 2009, Rahman et al. 2008).

There are other parameters that affect the importancy of a diagnostic test and are not affected by prevalence, namely the positive likelihood ratio (LR +) and the negative likelihood ratio (LR-). In this study, the LR + value was 4.78, which means that every 1 false positive result on the serum serology test the Immunochromatography method would get 5 (rounded up) true positive results. In general, a LR+ value of more than 10 has a good diagnostic value, so the higher the value, the better the ability of a test to detect a disease. This study obtained an LR- of 0.82, which means that for every 8 false negative test results, 10 true positive results will be obtained on serum serology. The lower the LR- value of a test, the better it is to detect a disease. Likelihood ratio - values below 0.1 are considered to have good diagnostic values. In this study, the LR+ values were not high, and the LR- values were not low so that the serum serology test using the immunochromatography method with CIM was not good for diagnosing a disease. A research using the same method in China had a higher LR+ value of 9.4 (Hung et al. 2002). Factors that affect false positive and false negative also affect the LR+ and LR-.

SpPIn (Specificity Positive In) and SnNOut (Sensitivity Negative Out) are known to help in enforcing or getting rid of a disease. Likelihood positive value is related to the clinical concept of rulling in a disease because this value will provide information on how much increased the probability of disease, if a positive test result is obtained. In contrast, the likelihood ratio negative value will provide information on how much the decrease in the probability of disease, if a negative test result is obtained. Then the LR- value is related to the clinical concept of rulling out a disease (Parikh et al. 2009). Specifity positive in shows that if the results are a very specific examination (high Sp) and very low LR- (<0.05), then the test results give positive results, meaning it is very good to establish the diagnosis. Furthermore, the examination shows that the patient is most likely to suffer (rulling in) the disease. Conversely, SnNOut shows that if the results are a very sensitive examination (high Sn) and a very high LR+ value (>20), then the test results give negative results, thus the examination succeeds in getting rid (rulling out) the existence of a suspected disease (Sackett et al. 2006). In this study, a high specificity (95.3%) was obtained but it was not supported by a low LR- (0.82), indicating that if positive results were obtained on serum serology examination using immunochromatography method with CIM, it was not good enough in diagnosing (rulling in) infection *H. pylori*. In this study, the sensitivity was low, and the LR+ value was not high. Thus, if a serum serology tool produces a negative test result, the test may not necessarily rule out the presence of *H. pylori* infection. Furthermore, this serum serology tool cannot be used for diagnosis of *H. pylori* infection in the study population and clinical importancy is not achieved (Tumbelaka 2016). The results of this study obtained an accuracy of serological examination tools with a CIM of 82.7%. The results of this study were consistent with previous report which showed an accuracy of serological examination with a CIM of 89% (Rahman et al. 2008).

CONCLUSION

Serological examination of the immunochromatography method with CIM still needs other testing tools to establish the diagnosis of *H. pylori* infection today. Overall serum serology examination using immunochromatography method with CIM cannot be used to rulling in or rulling out the diagnosis of *H. pylori* infection at this time in adult patients in the Endoscopic Unit of Dr. Soetomo General Hospital, Surabaya. Serum diagnostic tool of immunochromatography method with CIM could not subtitute histopathology to diagnose *H. pylori* infection.

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