

# Comparison of the accuracy of helicobacter pylori stool antigen (HPSA) test with campylobacter like organism (CLO) test in diagnostic of helicobacter pylori gastritis in children

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## Abstract

This study aimed to find the accuracy of the HPSA test with the CLO test in diagnosing helicobacter pylori gastritis in children. The cross sectional study conducted on 57 children with gastritis in October 2019 up to May 2020. Subject were children between two until eighteen years old who met the inclusion and exclusion criteria. HPSA examination was performed on the patient's stool sample using rapid immunochromatographic monoclonal antibody test for qualitative detection of H. pylori bacteria in human feces. From 57 samples, there were 37 children positively infected H. pylori. There were no difference in characteristics in the study population. The diagnostic value of the HPSA test showed a sensitivity and specificity of 83.8% and 95%. The positive and negative predictive value are 96.9% and 76% (PPV and NPV) with positive and negative likelihood ratio (LR + and LR -) are 16.76% and 0.17%, and an accuracy value of 87.7%.

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## Introduction

Helicobacter Pylori (H. pylori) bacteria is one of the most common bacteria infecting humans and is one of the gram-negative bacteria that has polar flagella, generally living on the stomach surface. H. pylori infection can cause chronic gastritis, peptic ulcer disease, laryngeal cancer, gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma in humans worldwide. The prevalence of H. pylori infection in developing countries is 70-90%, while in developed countries is 40-50%. Epidemiological data shows that 50-70% of people in several Asian countries, such as Korea, Turkey, Vietnam, have been detected exposed to H. pylori bacteria. Likewise, the population in Shanghai is 73.3%, Brazil's population is 63.2% in women and 66.5% in men (Uwan et al., 2016).

The diagnostic enforcement of H. pylori infection becomes a consideration for the patients as not all diagnostic tests are easy, low cost, even not all health centers have adequate equipment. In principle, the diagnostic test for H. pylori grouped into invasive and non-invasive methods (Sabbagh et al., 2019). Invasive

tests include tests that are done through an endoscopic examination, where there are four tests that are performed through endoscopy to diagnose *H. pylori* infection, namely the campylobacter like organism (CLO) test or known as the rapid urease test (RUT), gastric tissue biopsy (histopathology), culture and polymerase chain reaction (PCR). The meant of non-invasive test is the urea breath test (UBT), stool antigen test / *helicobacter pylori* stool antigen (HPSA), serology or antibody test. Whereas endoscopic examination is a prerequisite for all invasive methods and creates difficulties for children because it is a difficult procedure and requires patient cooperation (Darnindro et al., 2013).

Non-invasive tests have been commonly used in children, although their accuracy is not as good as invasive procedure they are still reliable in some cases, and some have low sensitivity and specificity in children (Sabbagh et al., 2019). Invasive tests may be selected to confirm the diagnosis when and when needed and are expensive compared to non-invasive methods of *H. pylori* infection. This is very high in developing countries where it is closely related to socioeconomic conditions and environmental hygiene and a gastric tissue biopsy culture is the gold standard diagnostic test against *H. pylori* infection (Hegar, 2000).

Therefore, this study intends to present the accuracy of the diagnostic tests used to detect *H. pylori* infection in children by focusing on the *Helicobacter Pylori Stool Antigen* (HPSA) test and CLO examination through endoscopy (Sabbagh et al., 2019). HPSA self-testing is a non-invasive method that has sensitivity and high specificity and relatively affordable among other non-invasive procedures and easy to implement. Meanwhile, the CLO test method, although it has high specificity and sensitivity as an invasive measure, it costs more, and not all health centres have adequate equipment (Hegar, 2000). This study still has limitations. First, comparisons of the HPSA test with other gold standards such as culture and histopathology were not performed, resulting in a lack of variation number in comparing to the accuracy of the HPSA test. Second, the HPSA test was not performed after eradication therapy so this study cannot explain the accuracy of the HPSA test in eradicating *H. pylori* in children.

## 1. Methods

### 1.1. Subjects and Methods

This study is a diagnostic study with a cross-sectional study approach. The research was conducted at H. Adam Malik (HAM) General Hospital, Universitas Sumatra Utara (USU) Hospital. The study was conducted from October 2019 to May 2020. This study involved 57 children with the inclusion criteria for outpatient and hospitalized patients who had a history of recurrent abdominal pain aged 2 years to <18 years. The exclusion criteria in this study are experiencing absolute contraindications to endoscopy including bowel perforation and relative contraindications, bleeding disorders and / or platelet disorders, neutropenia, patients at risk of perforation. Patients used drugs such as proton pump inhibitors (PPIs), H2 blocker antagonists, antibiotics and / or NSAIDs in the last 14 days. There are conditions that can affect the gastric mucosa, including patients who have a history of gastric surgery, gastric bleeding, cirrhosis of the liver, kidney failure requiring dialysis, heart failure with various complaints, early or advanced gastric cancer.

All research subjects were asked for consent from parents after prior explanation referring condition of the disease experienced and the examinations that had been carried out. Afterwards, the patient is asked for information for baseline patient characteristics. CLO examination performed through endoscopy was carried out with patients should not take antibiotics, bismuth and PPIs 2 weeks prior to biopsy (Sabbagh et al., 2019). It is recommended that a biopsy be performed in the gastric area approximately 2 cm from the lower pylorus or across the antrum arch. Cut the tissue that looks normal, avoiding eroded tissue as *H. pylori* is only present in small amounts in the area. Standard biopsy forceps should be performed according to the size appropriate for the test (Hospital Consolidated Laboratories).

Additionally, the sample can be properly fed into the CLO test stand. It is not justified to contaminate the specimen with blood in the first biopsy site. After removing the test slide from the refrigerator, remove the label on the CLO test slide in order to expose it to yellow gel (to expedite the test results, the gel should reach room temperature before insertion of the biopsy about 7-10 minute). Using a clean

applicator (eg toothpick) push the entire sample from the forceps under the surface of the gel, ensuring that the specimen is completely submerged. Put it back and cover with the label stamped, including the date and time of the specimen on the label. Place the specimen over the designated area to await reading. Dispose of specimens in a suitable biohazard container and document them. Any discoloration of the gel (red, magenta, pink, light orange) indicates the presence of *H. pylori* (positive result). Positive results should be recorded as quickly as possible. The negative test remains yellow after the tissue sample is set in the gel (if the gel color is yellow within 24 hours). If it is not read within 24 hours, store the slides at room temperature and read until 72 hours of storage (Hospital Consolidated Laboratories).

HPSA test was performed immunochromatographic technique for qualitative detection of *H. pylori* bacteria in human feces. The kit uses a specific monoclonal antibody against *H. pylori* antigen. During the test, the stool sample will react to red latex particles coated with the anti monoclonal *H. pylori* antibody. In the case of positive samples, the particles of the antigen-antibody complex migrate along the membrane coated with the anti *H. pylori* monoclonal antibody to the test line region. The specific antibody reacts with the antigen antibody particle complex and creates a red line in the T-line area. The green line control indicates that the test migration was performed well. Sample collection is done by taking the sample that has been provided with a paper mat. Prepare a tube from the kit provided, put dirt into the tube. Then, drop the dirt in the tube into the HpSA kit provided. Samples can be read after 10-15 minutes (Briand et al. 2015).

### 1.2. Research Ethics

This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Sumatera Utara / H. Adam Malik General Hospital in Medan No: 902/TGL/KEPK FK USU-RSUP HAM/2019.

### 1.3. Statistical Analysis

Data was statistically analyzed using SPSS software version 23.0. Descriptive statistics were expressed in the form of mean  $\pm$  standard deviation (SD) for normally distributed data. Data categories are presented in the form of frequency and proportion. Diagnostic test values are expressed in terms of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR +), negative likelihood ratio (LR-) and test accuracy value.

## 2. Result

This study was attended by 57 children with gastritis symptoms who had met the inclusion and exclusion criteria. There are 39 female children (68, 4%). The mean age of children involved in this study was 12 years with the youngest 2.8 years and the oldest 17.8 years. The mean body weight and height of the subjects were 38.35 kg and 138.04 cm. The largest ethnic group is the Batak tribe, amounting to 22 people (38.6%). The number of children with primary and junior high school education was 16 (28.1%) respectively. The majority of the subject's father and mother's education was tertiary institutions, amounting to 49 people (86%) and 52 people (91.2%), respectively. Generally, the socioeconomic level of the subjects was good as many as 50 people (87.7%). A total of 39 children (68.4%) had a family history of gastritis. The most clinical symptom in subjects was abdominal pain as many as 32 people (56.1%), followed by vomiting symptoms by 12 people (21.1%). Subject characteristics can be seen in table 1.

Table 1. Characteristics of Research Subjects

Subject Characteristics	n = 57
Gender, n (%)	
Male	18 (31.6)
Women	39 (68.4)

Age, n (%)	
Mean $\pm$ SD	12.06 $\pm$ 3.89
Weight, kg	
Mean $\pm$ SD	38.35 $\pm$ 14.61
Height, cm	
Mean $\pm$ SD	138.04 $\pm$ 19.87
Term, n (%)	
Aceh	5 (8.8)
Batak	22 (38.6)
Java	6 (10.5)
Malay	12 (21.1)
Minang	7 (12.3)
Papua	2 (3.5)
Chinese	3 (5.3)
Children's Education, n (%)	
Kindergarten	5 (8.8)
SD	16 (28.1)
Junior High	16 (28.1)
High school	13 (22.8)
College	7 (12.3)
Father's education, n (%)	
High school	8 (14)
College	49 (86)
Mother's Education, n (%)	
High school	5 (8.8)
College	52 (91.2)
Socioeconomic, n (%)	
Less	7 (12.3)
Good	50 (87.7)
Family History, n (%)	
Yes	39 (68.4)
No	18 (31.6)
Smoker father, n (%)	
Yes	35 (61.4)
No	22 (38.6)
Clinical Symptoms, n (%)	
Nausea	2 (3.5)
Gag	12 (21.1)
Vomiting blood	11 (19.3)
Stomach ache	32 (56.1)

The results of examining the presence of helicobacter pylori in all study subjects showed that there were 37 children (64.9%) positive with the CLO test and 32 people (56.1%) with the HPSA test. Meanwhile, there were negative results in 20 children (35.1%) from the CLO test and 25 people (43.9%) from the HPSA test. The results of examining the presence of H. pylori can be seen in Table 2.

Table 2. Examination Results for the presence of H. pylori

H. pylori examination results	n = 57
CLO	
Positive	37 (64.9)
Negative	20 (35.1)
HPSA	
Positive	32 (56.1)
Negative	25 (43.9)

Table 3. Diagnostic Value of HPSA Test in H.pylori Examination

HPSA	Sensitivity	Specificity	PPV	NPV	LR (+)	LR (-)	Accuracy
Positive	83.8%	95%	96.9%	76%	16.76	0.17	87.7%
Negative							

Table 3 shows the accuracy value of the HPSA test in diagnose helicobacter pylori infection in children. The sensitivity value of the HPSA test was 83.8%, the specificity value was 95%, the positive predictive value (PPV) was 96.9%, the negative predictive value (NPV) was 76%, the positive likelihood ratio (LR +) was 16.76, negative likelihood ratio (LR -) of 0.17 and an accuracy value of 87.7%.

### 3. Discussion

H. pylori infection is a disease that is acquired early in childhood and usually persists without antibiotics. The incidence of H. pylori infection is about 20% of the population in developed countries and about 90% of the population in developing countries, with primary oral-oral, fecal oral transmission (Calik et al., 2016). In Mexico about 30% of children 1 year of age and younger are known to have H. pylori colonization, so there is an increase of up to 50% before the age of 10 years (Montoya et al., 2017).

A study conducted by Hasosah (2019) in Saudi Arabia reported that 49.83% of children were positive for H. pylori infection, with the largest age group > 10 years as much as 57.76% and different results obtained in the study conducted by Mohsen, et al. (2018) in Iraq reported 33.3% positive H. pylori infection with the most age group 10-13 years as much as 40%. Study with a large sample size by Kakiuchi et al. (2019) In Saga Japan, 1,014 children reported that 78.5% of them were positive for H. pylori infection in 2016.

In this study, 57 gastritis children were examined at the RSUP. H. Adam Malik, RS. USU and network hospitals found 37 children (64.9%) positive for H. pylori infection. The mean age in this study was 12 years. The prevalence of H. pylori infection in this study was higher than the study conducted by Supriatmo (2007) in Medan in 2005 - 2007 on 41 children. In this study, 4 children with H. pylori infection were found (9.7%).

The diagnosis of H. pylori infection in children with gastritis is performed by invasive and non-invasive tests. All tests that are performed have the advantages and limitations of each test. The choice of CLO test as the gold standard in this study is because the CLO test is an invasive test that has high sensitivity and specificity, is fast to use and is cheap compared to other invasive tests. The HPSA test performed in this study is a non-invasive test, the advantage of the HPSA test is related to affordable but high in sensitivity and specificity among other non-invasive tests (Calik et al., 2016).

In this study, the examination of the presence of H. pylori which had a positive number of H. pylori positively found on the HPSA test was 56.1% and 43.9% negative. This number is almost comparable to the CLO test as the gold standard which has a positive number of H. pylori as much as 64.9% and 35.1% negative. The results obtained were not much different from the study conducted by Hasosah (2019) the number of positives on the HPSA test was 68.8% and the number of positives on the CLO test was 87.4% which was carried out on the examination of 151 children.

Test HPSA diagnostic test in the H. pylori examination with a 2 x 2 table showed a sensitivity of 83.8%, a specificity of 95%. The sensitivity value of 83.8% shows the results of the ability of the HPSA test to detect H. pylori infection in children of 83.8%. The 95% specificity value shows the results of the HPSA test's ability to show a negative value for children who are not infected with H. pylori by 95%. A study

conducted by Iranikah et al. (2013) reported the HPSA test sensitivity was 93% and 85 % specificity that was performed on 103 children in Iran. The data obtained in this study have the same value as the data in this study where there are high sensitivity and specificity values. The high sensitivity and specificity of the HPSA test as a non-invasive test indicates a high accuracy in establishing and screening *H. pylori* infection in children with gastrointestinal symptoms.

In a meta-analysis study conducted by Zhou et al. (2014) regarding the accuracy of HPSA in the diagnostic of *H. pylori* infection in children, the sensitivity and specificity values of 92.6% and 93.8% were reported before treatment and also the sensitivity and specificity values after eradication therapy were 80.9% and 97.2%. The data of this study show high sensitivity and specificity values. There are several factors that affect this condition; patient compliance in disease examinations including compliance with indications before the HPSA test, stool samples that are easy to obtain without the influence of media that can affect the results and samples can be frozen, and antigen detection that can be used for successful eradication therapy.

Although several other studies have been known to have a high diagnostic value of HPSA tests, there is a study conducted by Hasosah (2019) in Saudi Arabia that reported low sensitivity and specificity of HPSA tests of 69% and 73% in establishing *H. pylori* infection in children. The reason for the low sensitivity and specificity values in the HPSA test is caused by the difference quality variation of the tools used and not using standardized and validated tools in all available laboratories.

Studies conducted by Calik et al. (2016) comparison of HPSA test with CLO test reported PPV HPSA test of 97.02% and NPV 61.9%. Another study by Hunt et al. (2011) It was reported that the PPV HPSA test was 84% (Hunt et al., 2011). The data in the study showed a good PPV value and the data was the same in this study, it was found that the PPV HPSA test was 96.9% and NPV was 76%. The PPV 96.9% in this study showed that the HPSA test was truly positive in children with *H. pylori* infection at 96.9%. 76%. NPV in this study showed a completely negative HPSA test among 76 % children who are positively infected by *H. pylori*.

Measurement of the LR (+) and LR (-) of the HPSA tests in this study were 16.67 and 0.17 which indicated that the HPSA test had a good overall diagnostic value (LR (+) > 10 and LR (-) around 0.118 (Dahlan, 2018)). The accuracy value obtained was 87.7% indicating the proportion of correct HPSA test results among all pediatric patients who were examined

#### 4. Conclusion

The HPSA test can be used as an alternative test to uphold the diagnosis of *H. pylori* infection in regional service centers that do not have invasive diagnostic tools, on the basis of the advantages of the HPSA test is high in sensitivity and specificity, including non-invasive measures, relatively cheap prices, and easy to do. It is necessary to perform an HPSA test after therapy for the eradication of *H. pylori* infection in children to assess the accuracy of the HPSA test in eradicating *H. pylori* infection in children. Further studies with larger sample sizes and better study designs in other sites needs to be carried out on the accuracy of the HPSA test in *H. pylori* infection in child.

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