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Comparative evaluation of stool antigen immunoassay and blood antibody test methods for the screening of *helicobacter pylori* infection in symptomatic population at tertiary care hospital

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Abstract
Helicobacter pylori infection is a common cause of various gastrointestinal symptoms, including dyspepsia, abdominal pain, and nausea. Accurate and timely diagnosis is crucial for effective management of symptomatic individuals. This research paper aims to assess and compare the diagnostic performance of stool antigen immunoassay and blood antibody tests for the screening of *H. pylori* infection in symptomatic patients seeking care at a tertiary care hospital. A diverse population of symptomatic individuals will be enrolled in the study, and both test methods will be administered, with their results compared against a reference standard, such as endoscopy and histopathology. The research findings will provide valuable insights into the optimal diagnostic approach for identifying *H. pylori* infection in symptomatic patients, aiding in early intervention and improved patient outcomes.

Keywords: *H. pylori* infection, population of symptomatic, Immunoassay

Introduction
In light of the growing number of cases of *H. pylori* infection in India, there is a pressing need for basic research on the precision of diagnostic methods that are both financially feasible and easily accessible without causing undue discomfort. In this study, we compared the *H. pylori* infection between a stool antigen test and a blood antibody test strategy IgG. Our goal was to determine which method is a more effective and trustworthy painless test for the recognition of *H. pylori* infection in asymptomatic adult patients' population in India. Specifically, we looked at patients who were living in that region. In addition, the current study determined the exactness, responsiveness, explicitness, positive and negative probability proportions of the stool antigen and IgG serology tests, as well as variations in the rate of *H. pylori* infection based on gender, age, and geographical location. In short, our aim is to investigate the spread of *H. pylori* infection among asymptomatic and symptomatic subject of stool *H. pylori* antigen (HpSAg) and blood antibody (IgM) test [1].
This is because they can't differentiate between *H. pylori* infection in the past and *H. pylori* infection in the present. Stool antigen testing is performed in order to identify any traces of *H. pylori* antigens that may be present in the faeces. It is a reliable and accurate test for analyzing the *H. pylori* infection and confirming that it has been fixed after treatment. This is because it avoids recognizing a previous *H. pylori* infection, which makes it a reliable and accurate test. It is beneficial to the patients and may be easily carried out even in modest research facilities. Concerns arise, however, regarding the accuracy of its application in a wide variety of clinical settings and outside of controlled trials. In India, there is a dearth of research aimed at determining the accuracy of *H. pylori* symptomatic testing in the adult population who do not exhibit any symptoms of the infection [2].

Objectives
1. To determine the prevalence of *H. pylori* among Indians presenting with dyspepsia and controls at the Tertiary Care Hospital, India, using the stool antigen test.
2. To determine the prevalence of *H. pylori* among the same population by IgG /IgM serology.

Research Methodology
Biopsy Specimen
Ultra-rapid urease test (URUT)

H. pylori-contaminated stomach biopsy samples. When placed in a medium that contains urea, this will cause a shift in the pH level. The responsiveness of the test is dependent "on the number of microorganisms that are present in the sample, which may have implications for its use when evaluating treatment failures" and other related issues. The clarity is superb when the examination is read in one minute, but it gradually becomes less clear as more time passes throughout the hatching [3].

Olympus Videotrolley television z CLE-10 was used to collect the biopsy tissue after an Olympus GIF Type Q40 (2300903) camera was used. Every tissue obtained from the biopsy was immediately placed in a "capped Eppendorf tube containing 0.5 milliliters of a freshly pre-arranged arrangement of 10% urea in deionized water. Two drops of phenol red at 1% concentration were added to the solution as pH indicators. The color of the arrangement shifted from orange to pink within the first minute, indicating a successful conclusion, which was proven by the change" [4].

Microscopy: After reviewing the results of the ultra-quick urease test (URUT), a biopsy was removed from the urea solution and engraved spreads were created by carefully relocating the sample using a hypodermic needle on a flawless glass slide. The slides were then examined under a microscope. After being exposed to air for drying, the engraving smear was then fixed in pure methanol. The methylene blue stain developed by Loeffler was used to color the engraved spreads. In order to locate the bent bacilli, the oil submersion objective was used to the examination of the slides [5].

Stool sample
Kit components

ELISA for the detection of *H. pylori* in faeces, using a commercial test kit Premier Platinum HpSA Plus®, Meridian Bioscience Europe, Italy. 48 samples were taken from each well. There were two packets put to use. Antibody-coated microcells, a positive control, a negative control, a test diluent, a wash support, a catalyst form, a substrate, a stop arrangement, move pipettes, a strip holder, a strip sealer, and some wooden stick tools are all included in this kit [6]

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Pre-assay controls and operations

1. An example of a stool was prepared in accordance with the depiction in "Figure No.1, which lays out the guidelines for the appropriate application of the *H. pylori* Ag extraction unit".
2. Visual inspection of the fluid components for the presence of detectable totals or particles was performed (to take out the chance of pollution). By sucking up a little volume of the chromogen or substrate using a clean and simple plastic pipette, the coloration of the substance may be determined (drab or bright blue). The suggestions made by the maker were used to inspect any remaining parts.
3. "The composition of the 20x concentrated wash arrangement was diluted with the suitable solvent in order to reduce its potency".
4. The calibrator set was disassembled into its individual parts.
5. After waiting for all of the components to reach room temperature (about an hour), the mixture was then vortexed to combine the ingredients.
6. The ELISA incubator was adjusted to a temperature of 37 degrees Celsius
7. "The ELISA reader was switched on approximately twenty minutes before the reading was performed".
8. instructions for usage of the product

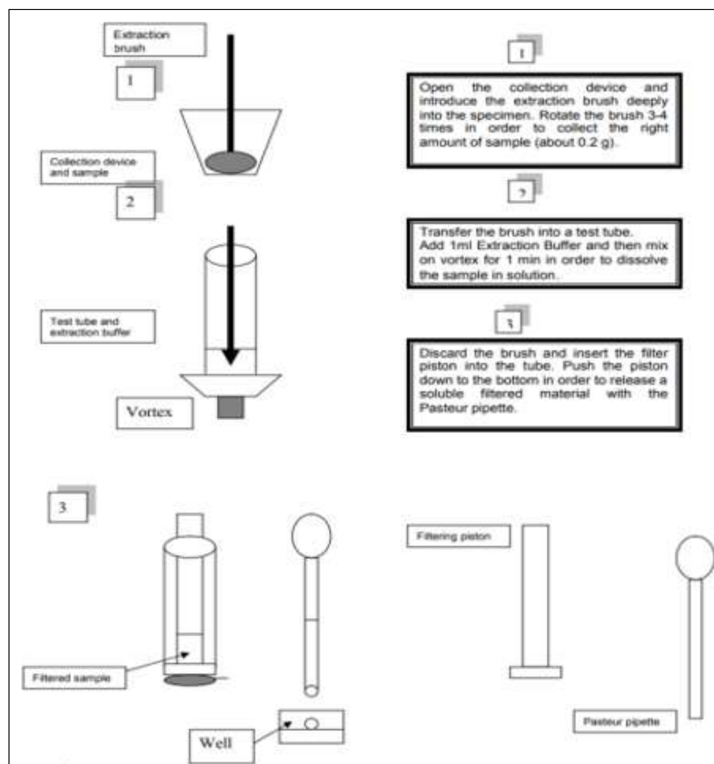


Fig 1: Schematic presentation of the HpSAg detection procedure"

Assay procedure (Quantitative Assay)

1. The needed quantity of strips was placed in the plastic holder, and the wells that were to be used for the calibrator and the tests were given descriptive names. For the purpose of blanking, wells A1 and B1 were not filled in their whole.
2. A duplicate of one hundred microliters of calibrators was pipetted into each well of the calibrator.
3. Using the Pasteur pipette that was supplied, three drops of the separated stool test were suctioned and distributed across the several wells in the example plate.
4. After that, 100ul of the enzymatic form were distributed across the wells, with the exception of wells 5 A1 and 5 B1, which were used for the blanking procedures.
5. After the expansion of the form, the colour of the instances changed from brown to a light rose colour, and the microplate was baked at 37 degrees Celsius for a total of one hundred twenty minutes.
6. Once the primary hatching process had been completed, the microplate was washed a number of times.
7. Two hundred microliters of chromogen and substrate were applied to the wells in general, including wells A1 and B1. The microplate was hatched for twenty minutes at room temperature (18-24 degrees Celsius) while it was covered from light.
8. To halt the enzymatic reaction, one hundred microliters of sulfuric acid was pipetted into each of the wells "using the same pipetting technique as was used in step.
9. The amount of light blocked by the configuration was measured in each well by employing channels with a wavelength of 450 nm as well as channels with a wavelength of 620-630 nm

Calculation of Result

For qualitative reading

The following equation was used to compute the results of the test, which were based on the "OD450 nm value of the (CAL0) and the OD450 nm value of the CAL0.1 ug/ml". However, the results of the test were not completely set in stone. Cut-off equals (CAL0+CAL0.1) divided by two [7].

Questionnaire: For the purpose of gathering information from qualifying patients, a meeting poll was conducted. Free factors that were taken into consideration for the survey included orientation, weight, height, age, general health, drinking tea or coffee, "smoking, type of food consumed (Meat, fish, vegetables, and others), type of water consumed during adolescence and adulthood (Filtered, region, or well water), trash collection in the area, and indicators of socioeconomic status such as educational level, family income, type of housing (House, level, estate), water supply (indeed, district), sewage system, number of rooms.

Results and Discussion

The months of September 2019 through August 2021 were dedicated to the conduct of this study. During the period under consideration for "this study, 120 patients who were undergoing upper gastrointestinal endoscopy were interviewed, and" they responded to a few questions concerning their personal information and their way of life. Stool, stomach biopsy, and serum samples were collected throughout this process. There were only 89 patients total who gave any "of the three sample kinds" [8].

Table 1: Age and sex distribution of the study sample

Age Group	Sex				Total	
	Male		Female			
	No	%	No	%	No	%
13-20	1	16.7	5	83.3	6	6.8
21-35	37	78.7	10	21.3	47	52.8
36-50	11	50	11	50	22	24.7
Over 51	8	57.1	6	42.9	14	15.7
Total	57	64.0	32	36.0	89	100

The ages of the people who participated in the research varied from 13 to 77, with a mean of 37.03 years. Of the total population, 36.0% are females and 64.0% are male. Male made up around two thirds of those in the age range of 21-35 years old. Table No.1 [9]

"Ultra-Rapid Urease Test"

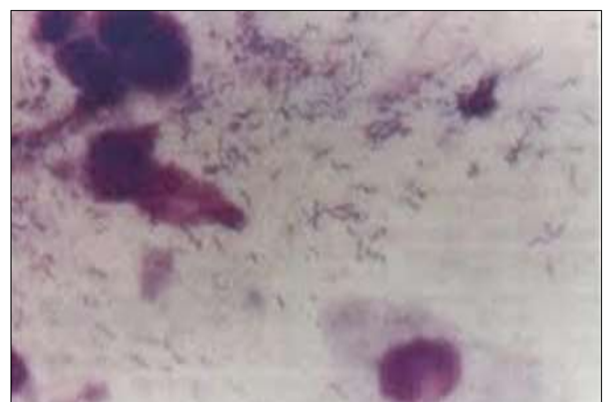
The examination that was carried out on the biopsy material collected from patients during upper gastroscopy turned out to be risk and uncomplicated. The results of the rapid urease test, which reveal that 29(32.6%) of the body's energy is being released, are displayed in the adjacent Both a positive (pink to red) and a negative (yellow to orange) URUT are depicted in Photograph No.1

Photograph 1: "Ultra rapid urease test (left positive; right negative)".

Gastric biopsies Stained with Methylene Blue

Even though it was simple to carry out and didn't require any special equipment, looking through the data and making sense of them was quite repetitive and took some time. 40 of the 86 samples that were stained with methylene blue revealed

H. pylori, which accounts for 46.5% of the during the course of this project, we failed to save three samples. A smear that was stained with methylene blue and shown to be positive for *H. pylori* is seen in Photograph No.2 [10].



Photograph 2: Methylene blue stained gastric biopsies showing the helical bacilli"

H. pylori Serum IgG

The results of an ELISA test done on *H. pylori* serum IgG are listed in the Table No.2 below. In accordance with the recommendations made by the manufacturer for deciphering the absorbances of serum tests, 40(44.9%) of the cases were considered to be definitive [11].

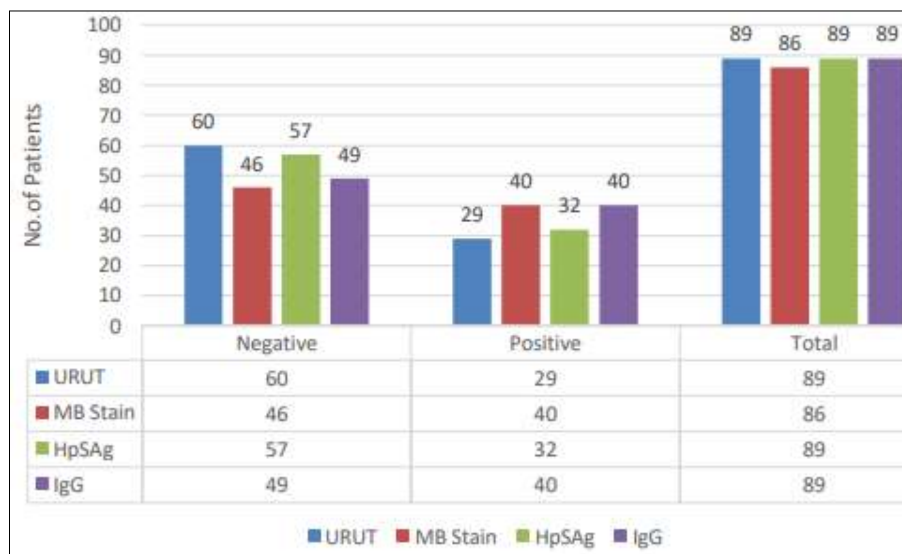
H. pylori antigen Detection from Stool"

"The finding of *H. pylori* antigen in faeces is receiving consideration from both administrative labs and medical professionals. According to the recommendation provided by the manufacturer for interpreting the absorbances of the removed stool samples, 32(36.0%) was considered to be certain Table No.2 [12].

Table 2: Distribution of positive and negative results in each of the four tests used

H. pylori	[1]URUT		[2]MB stain		[3]HpSAg		4IgG	
	No.	%	No.	%	No.	%	No.	%
Negative	60	67.4	46	53.5	57	64.0	49	55.1
Positive	29	32.6	40	46.5	32	36.0	40	44.9
Total	89	100	86	100	89	100	89	100

URUT; "Ultra Rapid Urease Test, MB; Methylene blue stain, [3] *H. pylori* stool antigen (HpSAg), and [4] *H. pylori* immunoglobulin G(IgG) were found. The following table illustrates the differences that can be found between each of the four tests: the most significant source of motivation for *H. pylori* was found in the methylene blue test 46.5% & IgG test with a 44.9% followed by the HpSAg test with 36.0% and the urease test with 32.6%. Graph No.1 [13].



Graph 1: Bar graph showing Distribution of positive and negative results in each of the four tests used

True Positive for *H. pylori* Infection"

As can be seen in Table No.3, there is a significant amount of variation in the proportion of successful results among the four applied tests. If both the URUT and the Methylene blue tests came back positive, one may assume that the result was accurate Table No.3. Each and every one of the ensuing associations between possible risk factors and *H. pylori* infection was completed using the real positive. Graph No.2 [14]

Table 3: True *H. pylori* positive

H. pylori	Frequency	Percent
Negative	46	51.7
Positive	43	48.3
Total	89	100.0

An Evaluation of the Results of Measurable *H. pylori* Tests (Chi-square) "There were statistically significant differences between MB and URUT, IgG, and HpSAg test results with a P-value of less than 0.01, as shown in Table No.4. The results of the MB and URUT tests are presented in which shows that the level of comprehension in some outcomes was 92.9%. "The IgG test and the MB presented a stark contrast, as shown in with a P value that was less than 0.01. There was a 69.2% likelihood of a favorable outcome according to the understanding. In the understanding between the MB and HpSAg test was 96.8% in a positive outcome, and in the understanding between the URUT and HpSAg test was 89.7% in a positive outcome; URUT and HpSAg gave factual huge contrasts with P=0.01; [15] however, MB and HpSAg gave positive results more frequently. In the difference between IgG and URUT was not significant (P=0.415). On the other hand, the difference between HpSAg and IgG that was exhibited in was significant with a P-value of 0.034 and an arrangement between a positive outcome of 47.5%".

Table 4: Chi-square test to determine whether there are statistically significant differences between the findings of the URUT and the Methylene Blue Stain.

Ultra-Rapid Urease Test (URUT)	MB Test				*P*- Value
	Negative		Positive		
	No	%	No	%	
Negative	44	75.9	14	24.1	<0.01
Positive	2	7.1	26	92.9	
Total	46.0	53.5	40	46.5	

Conclusion

It is also possible to assume that consuming water from an area or well that is contaminated with *H. pylori*, as well as making use of water from such an area, poses a risk of contracting *H. pylori*. Because *H. pylori* infection can have a negative impact on a person's overall health, it is important to take this into consideration while determining how far to go to avoid it. "According to the findings of this review, factors such as age, sex, weight, marital status, smoking, coffee consumption, oral cleanliness status, financial status, education level, pay, type of convenience, number of people living in the convenience, number of people in each room, type of water, the sewage system, contact with animals, travelling internationally, and drug use cannot be considered to be risk factors for *H. pylori* infection". "The factual investigation of material obtained from this work shown that tea had an important defensive function against *H. pylori* infection with a big P- value". The findings from this study lend credence to the hypothesis that *H. pylori* infections in non-industrialized countries are almost often acquired in adolescence or childhood. The prevalence of *H. pylori* in food controllers in India, 36 percent; this revealed a significant prevalence of *H. pylori* in asymptomatic food controllers in this area.

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Conflict of Interest: None

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