**Original article:**

# Utility of In House made Rapid Urease Broth Test for Detection of Helicobacter pylori Infection in Resource Constraint Settings

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**Abstract-**

**Introduction-** Helicobacter pylori (H. pylori) is a spiral, motile, gram negative bacillus, responsible for chronic gastritis, gastroduodenal ulcers and gastric carcinomas. Diagnosis of H. pylori is difficult as no single method is perfect. So generally combination of various tests is used for its diagnosis. Rapid Urease Test (RUT) is simple, low cost and rapid to detect H. pylori. Many commercial available RUT kits have been used earlier for the diagnosis of H. pylori. But its accessibility & cost usually restricts its use in resource constraint settings.

**Aim-**To evaluate the utility of in house made rapid urease broth test to diagnose H. pylori infection in resource constraint settings.

**Materials & methods-**A total of 50 patients with acid peptic disease were enrolled in the study. Diagnostic endoscopy was performed &two biopsy samples from gastric antrum were taken from each patient.A bit was transferred into the freshly prepared RUT broth immediately & another was rubbed on new slides for gram stain. Broth was observed initially within half an hour; then hourly for 4 hours and thereafter next day morning. Broth showing pink color was taken as positive.Presence of spiral or comma shaped bacilli were considered as positive for H. pylori in gram stain.Samples positive by both methods were considered true positive.

**Results-**Out of 50 samples, 17 were true positive (both RUT & gram stain positive). 4 samples were positive by RUT only. By comparing with gram stain; in house made rapid urease test showed 100 % sensitivity, 87.9% specificity, 80.9 % PPV & 100% NPV. Out of 21 positive samples by RUT,17 were positive within first half an hour to four hours; while 4 were positive only after 24 hours.

**Conclusion-**The present study concludes that in house rapid urease test is a rapid, simple test for H. pylori diagnosis. Also as other diagnostic methods are costly and difficult to perform in resource constrained settings, rapid urease test is good alternative. It is cheap & highly sensitive. The specificity of the test increases if it is interpretated within 1-4 hours of inoculation of sample.

**Keywords-** Helicobacter pylori, Rapid Urease test (RUT), Gram stain

**Keynotes-** In house Rapid urease test is a simple and cheap test to diagnose H.pylori infection if interpretated within 4 hours.

## Introduction-

Helicobacter pylori is a spiral, motile, gram negative bacillus, responsible for chronic gastritis, gastroduodenal ulcers and gastric carcinomas.[(1)] In India, Prevalence of H. pylori has been reported up to 80%.[(2)] H. pylori can be diagnosed by a wide variety of invasive & noninvasive tests. Invasive tests are- Rapid Urease Test (RUT), culture, histopathology,

PCR, imprint cytology. Noninvasive tests are serological test for IgG antibodies, urea breath test, stool antigen detection.[3] Also various stains like gram, Giemsa, Warthin Starry have been used for the demonstration of H. pylori in the clinical specimen.[4] Culture or histopathology are considered Gold standard techniques for diagnosis of H. pylori. But these are costly & time consuming. On the other hand, RUT is simple, low cost and rapid test to detect H. pylori.[4]Still in resource constraint settings, commercial RUT kits are costlier & have difficult accessibility. So it was decided to conduct the present study which will diagnose H. pylori by using in house made rapid urease test and gram stain which are economical. Evaluation of in house made RUT was done by comparing with the results of Gram stain.

**Materials & Methods-**

A total of 50 patients with acid peptic disease were enrolled in the study. Diagnostic endoscopy was performed after taking informed & written consent from all the patients. Two biopsy samples from gastric antrum were taken by consulting surgeon at our hospital. A bit was transferred into the freshly prepared RUT broth immediately in the endoscopy room. Rapid urease test broth was prepared as follows.

**Procedure of preparation of rapid urease test broth**–add 2.03g of rapid urease test broth powder (M1828) from Himedia Laboratories Pvt. Ltd. into 100 ml of sterile distilled water & mix well. Dispense 5ml aliquots into sterile test tubes. Quality control was done with each lot by using Proteus vulgaris &Klebsiellapneumoniae as positive control & E. coli as negative control.

All the samples in the broth were incubated at $37^0$C for 24 hrs. Broth was observed initially within half an hour; then hourly for 4 hours and thereafter next day morning. Broth showing pink color was taken as positive. (Fig 1&2.)

**Gram Stain Procedure-**

Second bit of biopsy was rubbed on new slides to prepare smears; air dried & heat fixed. Fixed smear stained with gram stain & observed under oil immersion. Presence of spiral or comma shaped bacilli were considered as positive for H. pylori.

The results of both RUT & gram stain were recorded. Samples positive by both methods were considered true positive.

**Results-**

Out of 50 samples, 17 were true positive (both RUT & gram stain positive). 4 samples were positive by RUT only. (Table No. 1) Prevalence of H. pylori was 34%.Accordingly, rapid urease test had 100 % sensitivity, 87.9% specificity, 80.9 % PPV & 100% NPV. Prevalence of H. pylori was 34%.Out of 21 positive samples by RUT,17 were positive within first half an hour to four hours; while 4 were positive only after 24 hours.



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**Table No. 1. Comparison of Rapid Urease Test with Gram stain**

| | **H. pylori positive** | **H. pylori negative** | **Total** |
|---|---|---|---|
| **Rapid Urease Test positive** | 17 | 4 | 21 |
| **Rapid Urease Test negative** | 0 | 29 | 29 |
| **Total** | 17 | 33 | 50 |

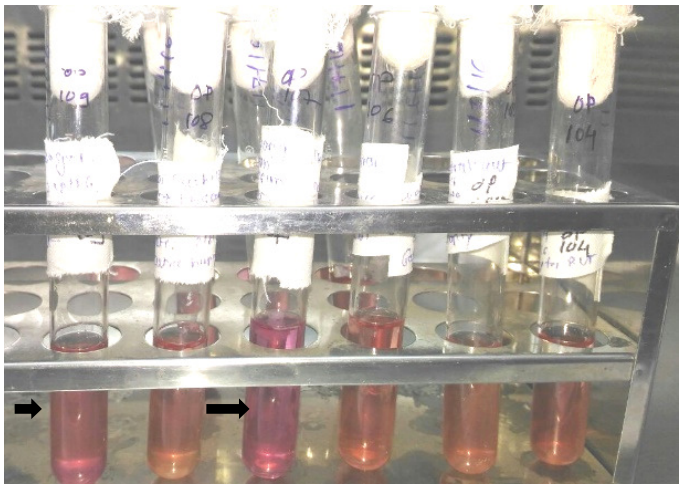

**Fig 1.** Positive Rapid urease test (arrows) in half an hour.

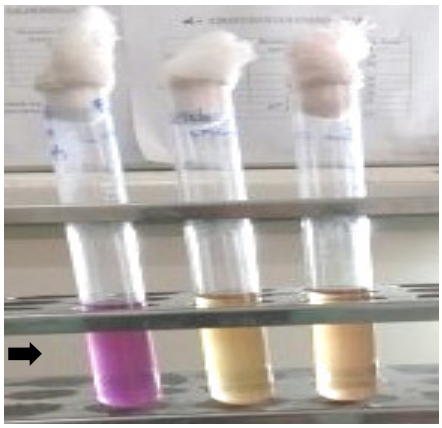

**Fig 2.** Positive Rapid urease test (arrow) in 4 hrs.



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**Discussion-**

Several tests are available for the detection of H. pylori infection. But each test has its advantages & disadvantages. Therefore, generally combinations of various tests are used for the precise diagnosis of H. pylori.

Though culture & histopathology are considered gold standard, they are difficult & time consuming & costlier than RUT. In our study, we used in house rapid urease test instead of commercially available RUT Kits. Evaluation of RUT was done by comparing the results with gram stain. Gram stain is a useful method for the diagnosis of H. pylori. It has 100% specificity with sensitivity in the range of 80-90%.[4,5,6]

In our study 34% patients were infected with H. pylori. Other studies had prevalence ranging from 2.9 % to 75.4%.[3,5,7,8]Low prevalence in our study may be due to geographic variation or false negativity of RUT. RUT gives false negative, if patient is on antibiotic, protein pump inhibitors or bismuth containing compounds which causes low bacterial load.[9]We have not taken into account these factors in our study.

Our study showed that in house rapid urease test had 100% sensitivity & 87.9% specificity. Many studies have shown similar results with high sensitivity & specificity.[4,5,7]

Also it was observed that accuracy of in house rapid urease test was maximum if the reading was taken within half an hour to four hours of collection. False positive rate increased with 24 hrs. reading. This clearly indicates that in house RUT should be best interpreted immediately (1-4 hrs.) & not after 24 hrs. It has another advantage of prompt reporting to the patient after endoscopy. Cohen H. et al [10] study compared three different RUT & concluded that time of interpretation of result is different with different RUT with equivalent accuracies.

The only limitation of present study was small sample size & no comparison with histology or culture. The studies with larger sample size along with comparison of in house RUT with gold standard methods are needed toconfirm ourfindings.

**Conclusion-**

The present study concludes that in house rapid urease test is a rapid, simple, cheap & highly sensitive test to diagnose H. pylori infection. It can be recommended in resource constrained settings where commercial RUT kits availability is difficult and costly. The specificity of the test can be improved by interpreting the results within 1-4 hours of inoculation of sample.

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