FRESHLY PREPARED RAPID UREASE TEST SOLUTION THE MOST SUITABLE TEST FOR HELICOBACTER PYLORI DETECTION IN OUR SET UP

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SUMMARY

Rapid urease test is a common diagnostic modality for the detection of helicobacter pylori. Despite the availability of many commercial rapid urease test preparations in the market, physicians face difficulty in accessing these due to various factors. We, therefore, have adapted to the method of preparing a fresh urease solution in our laboratory from the locally available ingredients. This solution was then compared to a commercial rapid urease preparation and histology for the detection of helicobacter pylori in the gastric biopsy specimens of the dyspeptic patients undergoing a diagnostic upper GI endoscopy. The solution was found to be simple to prepare, extremely cheap (2.98 Rs), and of high sensitivity (92.3%) and specificity (100%). We encourage the use of this solution on local level to help fight against this important organism.

INTRODUCTION

Helicobacter pylori i a common pathogen world wide, and the prevalence of this organism along with its associated diseases is significantly high in our local population as well.[1-3]

The diagnosis and eradication of this organism is a costly process, especially in a developing country like Pakistan and, therefore, physicians have to choose the cheapest and reliable test out of a battery of non invasive and invasive tests available for this purpose.[4] Rapid urease is a one such diagnostic test used because of its cost as well as reliability.[5,6]

Despite the availability of many commercial rapid urease test preparations in Pakistan, many physicians and endoscopist face difficuly in having access to these preparations.

Faced with a similar situation, we opted for the preparation of a freshly prepared rapid urease test solution in our local laboratory with the locally available ingredients by the method a described by Thillainayagam et al.[7] We then compared its specificity and sensitivity with a commercially available rapid urease preparation using histopathology as a gold standard.

MATERIAL AND METHODS

A fresh solution for the rapid urease test was prepared from the reagents as described in table-I. All working reagents were made up in HPLC grade water to avoid bacterial contamination. Appropriate reagent validation sheets were filled in and a batch number assigned.

In a plastic 30 ml pot 5.0 g of urea was weighed. It was then dissolved in 50 ml HPLC water using a small magnetic flea.

TABLE – 1

HELICOBACTER PYLORI QUICK UREASE TEST REAGENT

REAGENTS				
Urea	BDH	P 3	SP 10	
Phenol Red	BDH	P 2	SP 01	1 %(w/v)
Hydrochloric acid	BDH	P 3	SP 03	10 mMolar
Jackbean urease	Randox	P 3		10mg/l
Water(HPLC grade)	Ruthburn			

Whilst stirring 600 ul of phenol red solution was added to obtain a deep red solution.

Dropwise, 10mM HCL was added until the solution just turned yellow. pH was checked to be 6.0-6.8. 0.5 ml of the prepared solution was then pipetted out into 3 x test tubes / plastic tubes. Stopper were applied and the tubes stored at 4 degree Celsius. A positive control was included in each batch by pipetting 10 ul of the urease solution into one of the tubes. The test tubes were discarded if not used with in fourteen days. A commercially available rapid urease test preparation by the name of Helicourease (Department of immunology, Karachi University) was obtained to be used for comparison.

Sixty consecutive dyspeptic patients were entered in the study. Four biopsy specimens were obtained from the antral region of the stomach from each patient during an upper GI endoscopy. One specimen was put in the locally prepared fresh urease solution while the another was embedded in the helicourease test material according to the manufacture instructions. The remaining two specimens were transported to the laboratory in a formaline solution to be use for histo-pathologic assessment.

Taking the histopathology as the gold standard, individual sensitivities and specificities for the respective rapid urease test solutions were determined using the Epi Info WHO statistical software. Costs were compared for each case.

RESULTS

A total of 60 patients were entered in the study. Thirty-six were males and 24 females with an average age of 44.35 years (range 18 ---- 70 years).

Fifty-two (86.66%) patients demonstrated positive helicobacter on histopathology. The locally prepared fresh urease solution was positive in 48 patients. All of them showed a positive histology as well. Of the remaining 12 patients who were negative on this preparation, the histology demonstrated helicobacter pylori in 4 cases (false negative) while the remaining 8 remained negative on histopatology (true negatives). Compared to the fresh urease solution, the commercial preparation was positive for H.pylori in 49 cases (including the 48 on fresh solution). All of them had a positive histology for H. pylori as well. Out of the 11 negative patients on Helicourease, 3 were found to be positive on histopathology (false negatives). The sensitivities and specificities of the respective urease test are as given in Tables II and III.

The locally prepared solution was found to be the cheapest (Rs 2.98/-) compared to helicourease (Rs 25/-) and histology (Rs 400/-).

TABLE – II

HELICOUREASE TEST VS HISTOLOGY

		Histology	
		+	--
Helicourease Test	+	49	00
	--	03	08

Clinical parameter	Result	95% confidence limits
Sensitivity	94.2 %	83.1 % - 98.5 %
Specificity	100 %	59.8 % - 100 %
Positive Predictive Value	100 %	90.9 % - 100 %
Negative Predictive Value	72.7 %	39.3 % - 92.7 %

DISCUSSION

Initially considered as a medical curiosity, helicobacter pylori have now assumed a very important place in the world medical literature. A number of disease associations have been proved and many more on the verge of being nearly there[4,8,9]

Taking lead from the rest of the world, our local investigators have also published their work about the local trends of this important organism and its associated diseases. Studies from Karachi,[2,10] Lahore[11] and Peshawar[1] have shown a high prevalence of helicobacter pylori in our dyspeptic patients. Similarly, our own work has demonstrated a significant presence of peptic ulcer disease, gastritis and upper GI malignancy in patients under going diagnostic upper GI endoscopy.[3]

Despite the evidence presented by these studies, routine testing for helicobacter pylori has been hampered by many factors. Out of the non-invasive tests, urea breath testing is expensive, technically difficult and routinely not available in Pakistan. Few investigators have tried it at local level, but very few physicians have access to this

TABLE – III

FRESHLY PREPARED UREASE TEST SOLUTION VS HISTOLOGY

		Histology	
		+	--
Fresh urease Test solution	+	48	00
	--	04	08

Clinical parameter	Result	95 % confidence limits
Sensitivity	92.3 %	80.6 % - 97.5 %
Specificity	100 %	59.8 % - 100 %
Positive Predictive Value	100 %	90. 6 % - 100 %
Negative Predictive Value	66.7 %	35.4 % - 88.7 %

method of detection. Similarly, serological tests despite being non-invasive, less expensive and simple to perform are only recommended for large epidemiological studies.[4] However, it has been seen that they are currently being mainly used by the general practitioners in the peripheral health care facilities in Pakistan. Most of these general practitioners do not have access to diagnostic upper GI endoscopy, and they use the serological test for helicobacter detection only on presumptive diagnosis without confirming the underlying pathology. It can be argued that a big percentage of these patients might be suffering from non-ulcer dyspepsia or even irritable bowel syndrome and might not be candidates for eradication therapy at all. We, therefore, discourage the use of serological tests in such setting, as there is a danger of encouraging resistance to the various antibiotic used for helicobacter pylori eradication.

Histological confirmation of the helicobacter status with Heamatoxyline & Eosin and Geimsa stains is perhaps the most sensitive and specific way after culture. It can be a gold standard in our set up because of its easy availability as well as it authenticity. However, expense and delay in the availability of the result are two important draw backs for practical purposes, especially in view of the long distances which our patients have to travel before reaching a diagnostic upper GI endoscopic facility. It should be reserved for situations where the diagnosis can not be achieved by other methods or in borderline cases on other diagnostic tests.

In this back ground, rapid urease test assumes a great importance in our set up because of its simplicity, rapidity, good sensitivity and specificity and low costs. However, despite the availability of many commercial rapid urease preparations, we have always found difficulty in accessing these because of various factors. The demand is usually low, as the trend for routine testing for helicobacter has not picked up yet. Similarly, the profit to the manufacturer is not very high because of the low total price of the finished product. Majority of the preparations does not last for more than 6 months because of its unstability. All these factors result in the periodic disappearance of these commercial preparations from the market only to reappear after a long delay on the request, pressure and demand of an influential physician.

We have shown in this small study that a locally prepared fresh urease solution may be the most economical and easy way of overcoming this problem of non-availability of the commercial preparations.

The ingredients are locally available; preparation is simple and can be done by any local laboratory. It is extremely cheap and the results are comparable in sensitivity and specificity to other methods, and hopefully should encourage more frequent testing for this very important organism.

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