Detection of *Helicobacter pylori* infection by biopsy urease test

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Introduction

Several invasive and non-invasive methods (Table I) for diagnosing *H. pylori* infection have been developed since the bacteria was first isolated by Marshall and Warren in 1983 (1). Considered as "gold standard" for diagnosis of *H. pylori* infection, histology and culture have a major disavantage witch is the time required to get a definitive results. The desire to detect the infection rapidly led to the developement of numerous tests based on the high *H. pylori* urease activity. The rapid urease test — RUT — was introduced as a simple and a convenient method that often provides the endoscopist with a diagnosis so that therapy need not to be delayed until histology or bacterial culture results are avilables.

Table I. — **Different methods for detection *H. pylori***

Invasive methods	Non-invasive methods
— Urease test — Histology — Cytology — Culture — Molecular methods	— Serology — Urea breath test

Principle of the RUT

All strains of *H. pylori* produce a large amounts of urease (2), an enzyme witch the most purpose is to catalyse hydrolysis of urea to yield ammonia and carbonic acid :

$$\begin{array}{l} NH_2 \\ | \\ C=O + 2H_2O \longrightarrow 2NH_3 + CO_2 \\ | \\ NH_2 \end{array}$$

The ammonia is essential for the bacteria : it aids in colonization of the host by neutralizing gastric acidity and it is needed for bacterial protein synthesis. Also, urease elicits a strong immune response and induces directly host tissues dammage. In practice, a gastric biopsy specimen (it is preferable to perform 2 samples, 1 from the antrum and 1 from the corpus) is placed in a media containing urea and a pH indicator. If the gastric mucosa is colonized by *H. pylori*, the urease present hydrolyses urea with the production of bicarbonate and ammonia witch raises the pH. The pH change is detected by the pH indicator (phenol red) witch changes color from yellow to red.

Accuracy of different RUTs

Several commercially RUTs available are a variable of the original Christensen's medium used by McNulty (3) containing : urea, 20 g/l ; phenol red, 0.012 g/l ; KH_2P0_4, 2 g/l ; peptone, 1 g/l ; NaCl, 5 g/l ; glucose, 10 g/l. The aim of the modifications added to this initial medium was to increase the speed of the reaction and the sensitivity of the test (4). From the review of the literature of the 10 last years (about 70 studies concerning the RUT, number of patients varying from 40 to 1445), it appears that the sensitivity of the RUT is varying from 75% to 95% and the specificity from 95% to 100%. In all studies the "gold standard" diagnosis method of *H. pylori* infection was culture and/or histology. The CLO test (Delta West, Australia) is the most widely employed. In a serie of Marshall (5), sensitivity and specificity were ranging respectively from 75% to 95% and 95% to 100%. The recent RUTs (HUT test, Astra GmbH, Germany ; Pylorytek, Serim, Ind.) have a high accuracy, similar to the CLO test and have a significantly faster reaction time (6,7). The value of different RUTs is reported in Table II. The first generation of RUTs : Christensen's media, Modified Christensen's media (Christensen's media with high concentration of phenol red) have a good sensitivity but a relatively slow speed of reaction while the new generation tests seem to have a faster reaction (more than 90% within 1 hour). In Table III, is reported a comparison between a new reagent strip RUT (Pylorit)

Table II. — **Value of different biopsy urease tests**

	Sensitivity (%)	Specificity (%)	Speed
Christensen's modified	92	99.4	71% 2 hours
Christensens's	96	100	74% 2 hours
CLO test	89	98	92% 3 hours
CUT test	72	95	67% 3 hours
HUT test	90	100	75% 30 min
Pyloritek	99	95	90% 30 min
Hpfast	86	88	70% 30 min

Table III. — **Comparison between Pyloritek and CLO test for detection of *H. pylori* status**

		Se (%)	Sp (%)	PPV (%)	NPV (%)
Pyloritek	½ hour	93	100	100	86.5
	1 hour	98.5	97	98.5	94
	2 hours	100	97	98.5	100
CLO test	½ hour	93	100	100	86
	1 hour	93	100	100	86
	2 hours	94	100	100	86
	4 hours	97	100	100	94
	12 hours	97	100	100	94
	24 hours	98.5	100	100	97

Se : sensitivity ; Sp : specificity ; PPV : Positive predictive value ; NPV : Negative predictive value.

and an agar gel RUT (CLO test) : within 2 hours, sensitivity is 94% for the CLO test, 100% with the Pyloritek with an erroneous categorization in only 2.9%. The sensitivity of RUT depends upon the number of bacteria present in the biopsy specimen : 10.000 organisms are required for a positive results (8). The greater the number of bacteria is seen in biopsy section, then the more rapidly the test is positive (Table IV). It was also found a quantitative relationship between RUT reaction time and the grade of the chronic gastritis (Table V) (9). The analysis of 1 antrum and 1 corporeal biopsy specimen increases the sensitivity of the RUT compared with only 1 antrum biopsy. The size of the biopsy specimen does not seem to be important and warming the media is not necessary with the new generation of RUTs. Sampling in intestinal metaplasia, atrophic mucosa and adenocarcinoma area decreases the RUT sensitivity. Sensitivity of RUT decreases also in bleeding ulcer. After antimicrobial therapy, the sensitivity of the RUT decreases if the test is performed within 4 weeks after treatment, beyond 4 weeks, the sensitivity seems to be comparable to the histology (10) and culture (11). Lastly, sensitivity of RUT decreases under PPI treatment : PPI induce a direct inhibition of urease activity and reduce the number of viable *H. pylori* bacteria.

Conclusion

RUT is a simple, reliable and inexpensive test for diagnosing *H. pylori* infection. All commercially tests provide accurate results but the new generation tests are true rapid tests : more than 90% of *H. pylori* patients are identified within 1 hour. The RUT gives only answer to one question : presence or absence of bacteria. If more informations are needed (classification of gastritis, antibiogarams etc.) other methods are recommended (histology, culture etc.).

Table IV. — **Comparison of histological determination of bacterial number and biopsy urease test reaction time (%)**

Grade of bacteria	Urease reaction time (min)				
	0-30	30-60	60-120	> 120	Negative
0	1	2	1	10	86
1	44	12	14	16	14
2	84	6	3	6	1
3	100	0	0	0	0

Grade of bacteria : 0 : no bacteria seen ; 1 : occasionnal bacteria present ; 2 : intermediate bacterial number between 1 and 3 ; 3 : numerous bacteria in all fields.

Adapted from Hazell *et al. Am. J. Gastroenterol.*, 1987, vol. 82 (4), 292-296.

Table V. — **No. of biopsies giving specified urease reaction time compared to grade of chronic inflammation (%)**

Grade of inflammation	Urease reaction time (%)				
	0-30	> 30-60	> 60-120	> 120	Negative
0	0	0	1	6	93
1	34	13	13	10	30
2	68	68	5	11	11
3	72	17	0	11	0

Grade of inflammation : 0 : no mononuclear cells ; 1 : only occasional inflammatory cells present ; 2 : inflammatory cell infiltration intermediate between 1 and 3 ; 3 : very dense infiltration of inflammatory cells.

Adapted from Hazell *et al. Am. J. Gastroenterol.*, 1987, vol. 82 (4), 292-296.

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