A COMPARATIVE STUDY ON DIAGNOSTIC ACCURACY OF REUSED CLOtest® AND REUSED Pronto Dry® IN THE DIAGNOSIS OF *HELICOBACTER PYLORI* INFECTION IN HOSPITAL UNIVERSITI SAINS MALAYSIA (HUSM)

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Introduction: Various methods in *H.pylori* detection are available worldwide with different diagnostic accuracy. In theory, substrate that has not been consumed in a negative rapid urease test can be reused. We investigated the diagnostic accuracy between reused CLOtest® and reused Pronto Dry® in Hospital Universiti Sains Malaysia (HUSM), located in the North Eastern of Peninsular Malaysia with a known low prevalence of *H.pylori* .

Objectives: The aims of this study were to compare the diagnostic accuracy (sensitivity, specificity, positive predictive value, negative predictive value) between reused CLOtest® (Ballard Medical Products, Utah, USA) and reused Pronto Dry® (Medical Instrument Corporation, France) and To determine the

prevalence and factors associated with *H.pylori* infection among dyspepsia patients who undergo oesophageal-gastroduodenal scope (OGDS) in Hospital Universiti Sains Malaysia (HUSM).

Patients and Methods : A total of 410 adult patients with dyspepsia between March 2008 to June 2010 who underwent upper endoscopy needing rapid urease test on the basis of endoscopic findings were enrolled in this cross-sectional study. The gastric biospsies were tested for new Pronto Dry® , reused Pronto Dry® ,reused CLOtest® and histology. *H.pylori* infection was determined by either new Pronto Dry® or histology positive for *H.pylori* .

Results: Reused CLOtest® has higher sensitivity, specificity, PPV and NPV (93%,99%,97%,99% respectively) as compared to reused Pronto Dry® (72.6%,98%,93%,94% respectively). There was perfect kappa agreement between reused CLOtest® and new Pronto Dry®; 0.941(95%CI, 0.841-1.037). The overall prevalence of *H.pylori* among dyspepsia patients who underwent upper endoscopy in HUSM was 17.8%(73/410). Younger age group, Chinese ethnicity, underlying gastritis and peptic ulcer disease, and gastric ulcers and duodenitis on histology were strongly associated with *H.pylori* infection in multivariate analysis (p-value<0.05).

Conclusions: Reused CLOtest® is superior than reused Pronto Dry® on diagnostic accuracy. Reused CLOtest® can be considered to be used as a screening tool in the diagnosis of *H.pylori* infection in circumstances in which there are environmental and economic considerations to be taken into account.

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Thank you very much, may Allah bless us with peace, power and glory…Ameen

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ABBREVIATION

ACG	American College of Gastroenterology
BMI	Body mass index
DNA	Deoxyribonucleic acid
EHSG	European Helicobacter Study Group
GERD	Gastroesophageal reflux disease
HUSM	Hospital Universiti Sains Malaysia
MALT	Mucosa-associated lymphoid tissue
NIH	National Institutes of Health
NPV	Negative predictive value
NSAIDS	Non steroidal anti-inflammatory drugs
OGDS	Oesophageal-gastroduodenal scope
PAI	Plasminogen activator inhibitors
PPV	Positive predictive value
PUD	Peptic ulcer disease
RUT	Rapid urease test
UGIT	Upper gastrointestinal tract
WHO	World Health Organisation

ABSTRACT

Background: Various methods in *H.pylori* detection are available worldwide with different diagnostic accuracy. In theory, substrate that has not been consumed in a negative rapid urease test can be reused. We investigated the diagnostic accuracy between reused CLOtest® and reused Pronto Dry® in Hospital Universiti Sains Malaysia (HUSM), located in the North Eastern of Peninsular Malaysia with a known low prevalence of *H.pylori* . **Methods:** A total of 410 adult patients with dyspepsia between March 2008 to June 2010 who underwent upper endoscopy needing rapid urease test on the basis of endoscopic findings were enrolled in this cross-sectional study. The gastric biospsies were tested for new Pronto Dry® , reused Pronto Dry® ,reused CLOtest® and histology. *H.pylori* infection was determined by either new Pronto Dry® or histology positive for *H.pylori* .**Results:** Reused CLOtest® has higher sensitivity, specificity, PPV and NPV (93%,99%,97%,99% respectively) as compared to reused Pronto Dry® (72.6%,98%,93%,94% respectively). There was perfect kappa agreement between reused CLOtest® and new Pronto Dry®; 0.941(95%CI, 0.841-1.037). The overall prevalence of *H.pylori* among dyspepsia patients who underwent upper endoscopy in HUSM was 17.8%(73/410). Younger age group, Chinese ethnicity, underlying gastritis and peptic ulcer disease, and gastric ulcers and duodenitis on histology were strongly associated with *H.pylori* infection in multivariate analysis (p-value<0.05). **Conclusions:** Reused CLOtest® is superior than reused Pronto Dry® on diagnostic accuracy. Reused CLOtest® can be considered to be used as a screening tool in the diagnosis of *H.pylori* infection in circumstances in which there are environmental and economic considerations to be taken into account.

ABSTRAK

Latar Belakang: Pelbagai kaedah dalam pengesanan *H.pylori* terdapat di seluruh dunia dengan ketepatan diagnostik yang berbeza. Berdasarkan teori, substrat di dalam ujian urease cepat yang negatif boleh diguna semula. Maka dengan itu, kami mengkaji ketepatan diagnostik antara CLOtest® guna semula dan Pronto Dry® guna semula di Hospital Universiti Sains Malaysia (HUSM), yang terletak di Timur Utara Semenanjung Malaysia yang terkenal dengan kadar prevalensi *H.pylori* yang rendah. **Kaedah:** Seramai 410 pesakit dewasa dengan gejala dispepsia antara bulan Mac 2008 hingga Jun 2010 yang menjalani endoskopi atas yang memerlukan ujian urease cepat berdasarkan penemuan endoskopik, telah direkrut di dalam kajian hirisan lintang ini. Biopsi perut telah diuji dengan Pronto Dry® baru, Pronto Dry® guna semula, CLOtest® guna semula dan histologi. Jangkitan *H.pylori* ditentukan oleh keputusan positif Pronto Dry® baru atau histologi. **Keputusan:** CLOtest® guna semula mempunyai sensitiviti, spesifisitasi, PPV dan NPV (masing-masing 93%, 99%, 97%, 99%) yang lebih tinggi berbanding Pronto Dry® guna semula (masing-masing 72,6%, 98%, 93%, 94%). Terdapat kesepakatan kappa sempurna antara CLOtest® guna semula dengan Pronto Dry® baru; 0.941 (95% CI,0.841-1.037). Keseluruhan kadar prevalensi *H.pylori* bagi pesakit dispepsia yang menjalani endoskopi atas di HUSM adalah 17.8% (73/410). Melalui analisis multivariasi, kumpulan usia muda, etnik Cina, sejarah penyakit gastrik dan ulcer peptik, dan laporan histologi ulcer peptik dan duodenitis adalah factor-factor yang sangat berkait rapat dengan jangkitan *H.pylori* (p-value<0.05). **Kesimpulan:** Ketepatan diagnostik CLOtest® guna semula lebih unggul berbanding Pronto Dry® guna semula. CLOtest® guna semula boleh disyorkan untuk diguna-pakai sebagai alat saringan dalam mengesan jangkitan *H.pylori* di dalam situasi di mana pertimbangan faktor persekitaran dan ekonomi perlu diambil kira .

CHAPTER 1

INTRODUCTION

1.1 Study Background and Rationale

Helicobacter pylori (*H.pylori*) has been the subject of intense discussion since its culture from a gastric biopsy back in year 1982 by Barry Marshall and Robyn Warren. During that era, few if any gastroenterologist would have predicted that almost 20 years later, this bacterium would have been shown to be one of the most common bacterial infections responsible as the etiological agent for acute or chronic gastritis and firmly established as a predisposing factors for gastroduodenal disease, particularly peptic ulcer disease, gastric malignancies and B-cell mucosa-associated lymphoid tissue (MALT) lymphoma (Graham et al., 1992; Marshall et al., 1985; Parsonnet et al., 1994).

There are strong, positive correlations between gastric carcinoma rates and *H.pylori* infection rates in certain populations, and it has been classified as a Class I carcinogen by the WHO (WHO, 1994). True enough, this bacterium has provoked the interest and attention to bacteriologist, gastroenterologist, infectious disease specialist, cancer biologist, epidemiologist, pathologist and pharmaceutical scientist for further new scientific informations and development. Many updates from all over the world concerning this unique bacteria have been published in recent years including guidelines and consensus statement with regards to the treatment, screening tools and also bacteria virulence factors.

Detection of *H.pylori* is one of area of interest. Various diagnostic methods have been developed worldwide to detect *H.pylori* infection, either invasive or non-invasive. The invasive method includes upper endoscopic gastric biopsy tested for rapid urease test (RUT), histology and immunobiochemistry, culture and polymerase chain reaction. The non-invasive methods in the other hand are serology (serum IgG), urea breath test(UBT), urinary excreation of (15N) ammonia and stools antigen assay. There have been many studies comparing those detection methods merely to ensure superiority in terms of diagnostic accuracy and qualitative characteristics between them. New emerging method or new kit with similar principle affect and compete with currently available tests especially if better diagnostic accuracy offered.

However, up to recently rapid urease test (RUT) is the most widely used method for *H.pylori* detection merely due to easily available, affordable and more importantly acceptable diagnostic accuracy. Series of study have shown that RUT has persistently high sensitivity (95-98%) and specificity (92-100%)(Yakoob et al., 2006; Keeken et al., 2006; Chomvarian et al., 2005; Said et al., 2005; Chang et al., 2005;Loren et al., 1996). In Malaysia, most of gastrointestinal centers use rapid urease test (RUT) (either CLOtest® and Pronto Dry®) to detect *H.pylori*.

The RUT was originally designed as a disposable test for the rapid detection *H.pylori* before the patient leaves the endoscopy room. Based on the theory, substrate that has not been consumed in a negative rapid urease test can be reused, Lee et al., (1999) from Taiwan had conducted a study looking at feasibility of reused CLOtest®. The results were amazingly remarkable with high diagnostic accuracy

(sensitivity:98.6%; and specificity:98.2%). Not just that, they have proved negative CLOtest® pallets can be reused repeatedly within 6 months after initial usage provided the pallets are stored at room temperature (Lee et al., 2002). After a successful study by Lee et al., (1999), there were few series of study have shown similar remarkable results (Tomtitchong, 1999; Chong et al., 2007; Elitsur et al., 2001). Tomtitchong (1999) for example proved the feasibility of reused CLOtest comparing with new CLOtest with sensitivity, specificity, PPV and NPV were 100%, 97.4%, 95.8% and 100% respectively. He also demonstrated that the per cent error of reused CLOtest was low (4.35%) as compared to new test. These studies conclude that properties of the negative pallets were not affected by previous use. However there were no published data regarding diagnostic accuracy of reused Pronto Dry® and comparison on feasibility between reused CLOtest® and reused Pronto Dry® . Hence, this study would validate and compare the diagnostic accuracy and qualitative characteristic of these two reused RUT and perhaps could be recommended to be used as a screening tool for *H.pylori* detection.

Although RUT is not really an expensive item in developed countries, it may represent an extra medical expenses in developing or underdeveloped countries, either to the patient or to the health authority. Therefore, it will be benefit when it is possible to recycle a waste product to reduce financial burden.

1.2 Overview of *Helicobacter pylori* infection

1.2.1 Historical perspective of *H.pylori*

H.pylori was first discovered by a research registrar and histopathologist from Perth Australia, Robby Warren and Barry Marshall in 1982 where the bacterium was successfully cultured (Warren et al., 1983). The spiral bacteria that was biopsied from human gastric mucosa, initially identified as *Campylobacter pyloridis* and then *Campylobacter pylori* before taking its present moniker of *Helicobacter pylori* in 1989. The name was changed from *Campylobacter to Helicobacter* when it was discovered that its 16s ribosomal DNA did not have the characteristic sequences found in *Campylobacters* (Goodwin et al.,1993).

1.2.2 Epidemiological aspect of *H.pylori*

H.pylori is one the most common human infection wordwide **(Image 1)**. Approximately 50% of the world's population is estimated to be infected with *H.pylori*. Although infection occur worldwide, there are significant differences in the prevalence of infection both within and between countries (Goh KL, 1997; Malathy et al., 1992; Megraud et al., 1989). In general, the overall prevalence of *H.pylori* infection in developed countries is lower than in developing countries (Bardhan PK, 1997; Graham et al., 1991). Epidemiological studies suggested that the difference in prevalence of infection has been attributed to socioeconomic, ethnic socio-cultural, genetic, dietary, environmental factors, and the rate of acquisition of the *H.pylori* infection in childhood (Mitchell et al., 1992).

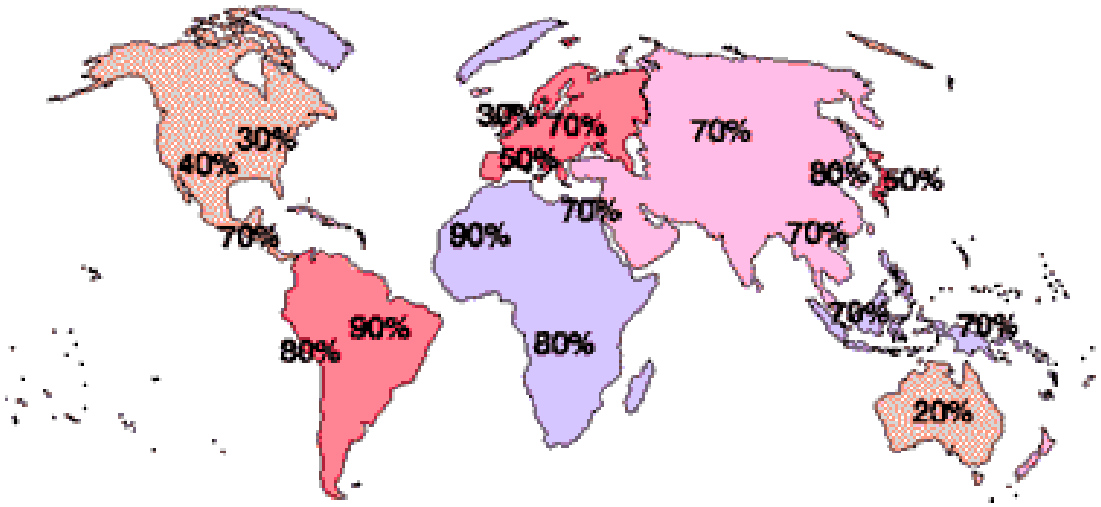

Image 1. Infection with *H. pylori* occurs worldwide, but the prevalence varies greatly among countries and among population groups within the same country.

(Photo courtesy of Comparative Bioinformatics Services (CBS), National Research Program for Genomic Medicine (NRPGM), Taiwan)

Taken from http://cbs.ym.edu.tw/cbs01/images/stories/cbs/digitallife/Epidemiology.gif

Series of epidemiological study have shown that poor socioeconomic status is strongly associated with *H.pylori* infection (Goodmann and Correa, 1997; Malaty and Evans, 1991; Sitas and Forman, 1991; Basso and Clive, 1990; Steward and Hodas, 1997). Socioeconomic status parallel with *H.pylori* rate of transmission (oral-oral and feacal oral) because of hygienic component is thought to be the main contributing factor. Goodmann and Correa, (1997) have reported *H.pylori* is more prevalent in

lower socioeconomic groups with poor living standard, such as crowded living conditions, poor sanitation and lack of clean water supply. Malaty and Evans (1991) also reported similar findings on low socioeconomic with low income in Arkansas. A comparable study was done in Wales where by the age adjusted prevalence of *H. pylori* was the highest in the lowest social class, lower in the middle class and the lowest in the upper class (Sitas and Forman, 1991). A higher prevalence of *H. pylori* infection was also seen among Irish soldiers with a low socioeconomic background (Basso and Clive, 1990). Another epidemiological study confirmed a high prevalence of *H. pylori* infection among an indigenous Indian population in the Andean mountains of Chile of 85%, compared to a 55% infection rate in the population living in urban Santiago (Steward and Hodas, 1997).

The assumption that high prevalence of *H.pylori* in low status of education has been confirmed by numerous of prevalence studies. Evan and Abdulghani, (1990) have reported high infection rate among non college graduate in Saudi Arabia, 77% as compared to those college graduate 54%. The interest on relationship between level of education and *H.pylori* infection was continued by EUROGAST study group (1993). It was a multicenter study enrolled 17 geographically defined populations in Europe, North Africa, North America, and Japan. The study shown *H.pylori* infection rate was lower among higher education level (34%), followed by secondary education (46%) and the highest in people received primary education (62%). Graham et al., (1995) also have reported high prevalence of *H.pylori* in those with low levels of education and poor hygiene. Education is actually an indirect index of hygiene facilities and practice that determine the tendency of transmission of *H.pylori*.

Cardiovascular disease (CVD) had been associated with chronic infection by *H.pylori*. This association may be partly due to an increase in metabolic risk factors for CVD such as high body mass index (BMI) (Ekesbo et al., 2000). However study by Wu et al., (2005) from Taiwan showed conflicting results. Wu et al., (2005) concluded that there was inverse relationship between morbid obesity and *H.pylori* infection. The study suggested colonization of the stomach by *H.pylori* might suppress gastric expression of strong appetite-stimulating hormone namely ghrelin. Hence, patient who have been cleared of the *H.pylori* infection would gain weight.

H.pylori infection is ubiquitous and infects both male and females (Goh KL, 1997; Malaty et al., 1992). Most of the epidemiological studies have shown the prevalence of *H.pylori* among both gender was the same. However, epidemiological reports by Goodman et al., (1996) showed higher infection rates in young male in rural Columbian Andes Community, New Zealand. This was also observed among some ethnic group in California where male population was more predominant (Replogle et al., 1995).

Infection with *H.pylori* is associated with chronic gastritis in children as well as in adults (Blaser, 1990). In children, *H.pylori* gastritis seems to be asymptomatic (Machartur et al., 1999; Bode et al.,1998). Many cross-sectional studies have demonstrated that higher prevalence of *H.pylori* infection is associated with increasing age in developed countries. In the West, a positive serology was uncommon in children, but present in about 20% of person under age of 40 years old and 50% of those over 60 years old (Graham et al., 1991). Seroprevalence in adults appeared to be independent of socioeconomic factors, but infection rates in children

showed an inverse correlation to education level and infection status of the mother. In developed countries, prevalence of *H.pylori* infection increases by - l% per year after adolescence (Graham et al., 1991; Perez-Parez et al., 1992), reaching a plateau of - 50% ± 20% by the seventh decade (Taylor et al., 1991; Megroud et al., 1989; Mitchel et al., 1992). It is unclear whether this steady increase in prevalence is due to a continued risk of acquiring the infection or to a cohort effect, in which an individual born in an earlier birth year had a higher risk of acquiring the infection than a person born in a later one .

Epidemiology study has reported that certain ethnic groups are more susceptible to *H.pylori* infection. There is great difference in its prevalence among different societies and ethnic groups, even within the same country. Overall, *H.pylori* prevalence in USA is estimated at 30-40% but it remains much higher in certain ethnic groups such as African-American and Hispanics (Replogle et al., 1995; Dehesa and Dooley, 1991). The overall seropositivity of *H. pylori* among African Americans was 57%, compared with 26% in the Whites, and this association was independent of age, gender,diet and rural or urban location (Hopkin and Russel, 1990). In New Zealand, seropositivity with *H. pylori* among adults varies by ethnic origin with prevalence rates of 70% among Tongans, 44% in Samoans, 39% in Cook islanders and 15% in Caucasians (Morris and Nicholson, 1986). A study that was done in Australia among immigrants that showed a wide variation in the prevalence of *H. pylori* infection: 43% in Ethiopians, 40% in Salvadorans and 18% in Vietnamese (Dryger and Kaldar, 1988). The prevalence of *H. pylori* was reported to be 0.5% among the Australian Aborigine population who has a low incidence of peptic ulcer

disease (Dwyer and Sun, 1988). This finding is similar to Malaysian aborigines where the prevalence of *H. pylori* infection is only 19% (Amry et al., 2010).

In Malaysia with multiracial (Malay, Chinese and Indian) population, prevalence of *H.pylori* is much higher among Chinese and Indian, 48.5% and 61.8% respectively as compared to Malay (16.4%) (Goh, 1997). This was supported by a study from a neighbouring country Singapore done by Kang et al., (1990) which also showed a higher prevalence among Chinese and Indian ethnic compared to Malays (Chinese 38%, Indian 35%, Malay 15%). However, the study by Goh, (1997) was not representing overall *H.pylori* infection in Malaysia population in view of the sample population was only among dyspeptic patients who underwent endoscopy at endoscopic Unit, University Hospital, Kuala Lumpur.

Study by Gurjeet and Naing, (2003) have reported that *H.pylori* infection in North Eastern region of Peninsular Malaysia showed high prevalence of *H.pylori* among non-Malays as compared to Malays. It was a retrospective study from pathology records that showed prevalence of *H.pylori* among Chinese and Indian population (24.1% and 28.6% respectively). *H.pylori* prevalence among Malays was 6.6%. The different races although living together, have exclusive habits and socio-cultural practices peculiar to their own that may be responsible for transmission of the infection besides possibility inherent genetic predisposition.

The overall prevalence of *H.pylori* in North Eastern of Peninsular Malaysia from Gurjeet and Naing, (2003) was only 13.5% (54 out of 400) based on positive *H.pylori* on histology. The low prevalence of *H.pylori* in this region was noted 10 years earlier by Uyub et al., (1994) when they did a seroprevalence study among 496

blood donor and 921 subjects who attended health screening clinics. The prevalence of *H.pylori* among blood donor and subjects attended health screening clinic in North Eastern of Peninsular Malaysia was very low 4.2% and 4.8% respectively. They also reported the incidence of peptic ulcer perforation (1991-1992) was 1.5 per 100 000 person per year.

The study population for both of the study however did not specifically targeted the dyspepsia patient who has higher incidence of *H.pylori*, the detection method for *H.pylori* used in Uyub et al., (1994) study was bacteria serology that have possibility of false negative. Serological methods for the detection of *H.pylori* organisms are the most commonly used technique for population based epidemiological studies but are less reliable (Varghese,2002). In our study, the study population was specifically targeted to those who had dyspepsia as a prerequisite criterion who underwent upper endoscopy. This would perhaps give clearer picture of prevalence of *H.pylori* in this region.

H. pylori infection is believed to be lifelong in most affected individuals (Taylor et al., 1991; Graham et al., 1991). Identical *H. pylori* strains have been isolated from serial endoscopic biopsies over several years in the same patients, and antibodies to the organism remain stable over time, only decreasing when the organism has been eradicated with antibiotic treatment (Kosunen et al., 1992; Langanberg 1988) . Data from the current study also showed that antibody titers were stable in most subjects, consistent with continued antigenic stimulation by the organism**.** *H.pylori* has difference strain that persists for years, decades, even for life. Molecular epidemiologic analysis indicates the strains themselves have strong linkage

to ethnic origins that can be traced back to the earliest known patterns of human migration. *H.pylori* has been called as “accidental tourist” which has established in the stomachs of humans thousands of years ago and remained bound to the original population as it dispersed from continent to continent.

1.2.3 **Microbiology and pathophysiology aspect of *Helicobacter pylori***

H.pylori has morphological and growth similarities to the *campylobacters,* with which they were originally classified. The cells are slender, curved rods with polar flagella **(Image 2).** The cell wall structure is typical of other Gram-negative bacteria. Growth requires a microaerophilic atmosphere and its slow (3-5 days).

H.pylori colonizes only gastric mucus-secreating cells, beneath the gastric mucous layers and surface fimbriae are believed to be one of adhesions associated with this process. *H.pylori* binds to Lewis B antigens. These antigens are part of the blood group antigens that determine blood group O. This findings account for the higher rate of ulcers in people with this blood type. *H.pylori* also binds to the monosaccharides sialic acids, which is found in the glycoproteins on the surface of gastric epithelial cells. Movement into the mucous layer may be aided by the fact that *H.pylori* is a strong producer of urease. Urease activity is thought to create a localized alkaline environment when hydrolysis of urea produces ammonia. The increase pH may protect the bacterium from gastric acid enabling it to grow under the layer of mucus in the stomach. The urease is produced in large amount (6% of bacterial protein) that its action can be demonstrated within minutes of placing *H.pylori* in the presence of urea. Another secreted protein called the vacuolating cytotoxin (VacA) causes apoptosis in eukaryocytes cells. It enters generating multiple large

cytoplasmics vacuoles. Vacuoles are felt to be generated by the toxin's formation of channel in lysosomal and endosomal membranes.

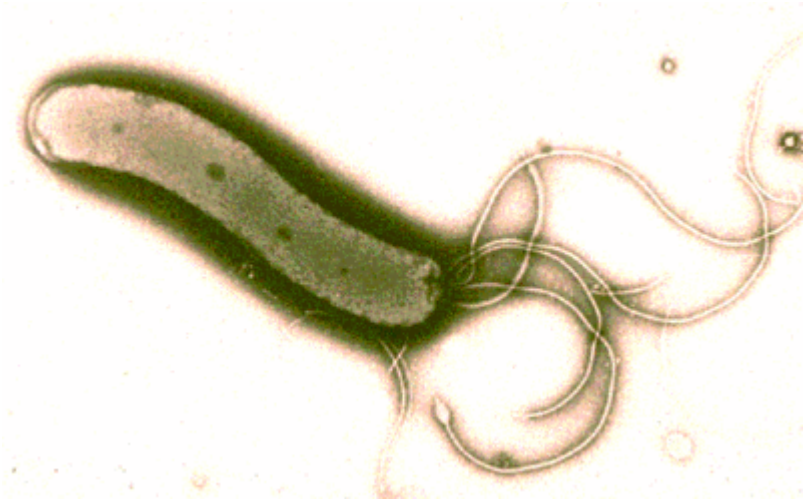

Image 2. Electron micrograph of *Helicobacter pylori*, flagellated, slender and curved rods bacterium.

(Photo courtesy of steadyhealth.com)

Photo taken from http://www.steadyhealth.com/4540/Image/H.pylori.gif

Most *H.pylori* strains also contain a PAI with 30+ genes, most of which code for elements of an injection secreation system. The secreation system injects VacA and a protein Cag, also coded in the PAI, into epithelial cells. Once in the cell, Cag induces changes in multiple cellular proteins and has a strong association with virulence that is responsible for epithelial cell damage and inflammation probably include proteases, phospolipases, cytokines and cytotoxins

Added together, urease, Cag and VacA provide ample explanation for the gastritis that is universal in *H.pylori* infection. Furthermore, colonization by *H.pylori* is almost always accompanied by a cellular infiltrate ranging from minimal mononuclear infiltrate of lamina propia to extensive inflammation with neutrophils, lymphocytes and microabcesses formation. This prolonged and aggressive inflamatory response could lead to epithelial cell death and ulcers.

1.3 Clinical manifestation of *Helicobacter pylori* infection and Gastric cancer risk.

When Warren and Marshall first identified spiral organisms closely applied to the gastric epithelium in active chronic gastritis, they brought to light an etiological explanation for a whole series of pathological changes that has been long recognized but not understood. It was widely appreciated that chronic gastritis was a common denominator linking peptic ulceration, gastric carcinoma and lymphoma and that histological picture encompassed chronic inflammation, atrophy and intestinal metaplasia. Primary infection with *H.pylori* is either silent or causes an illness with nausea and upper abdominal pain lasting up to 2 weeks. Years later, the findings of gastritis and peptic ulcer disease (PUD) include nausea, anorexia, vomiting, epigastric pain and even less specific symtoms such as belching that occurs intermittently or constantly (Tally et al.,1999). These constellations of symptoms are known as dyspepsia. Nevertheless, this symptoms is not exclusively referring to gastritis and PUD only but also include in the spectrum are gastric cancer, gastroesophageal reflux

disease (GERD) and functional dyspepsia. It has been suggested that up to 95% of duodenal and 70% of gastric ulcers are attributable to this infection.

Those who are at high risk of developing cancer often present with additional signs and symptoms, so called 'alarm symptom'. Therefore, provisional diagnosis based on history and physical examination alone are often inaccurate; leading to inappropriate management plans and/or a delay in establishing correct diagnosis (Westbrook et al.,2001).

The 'alarm symptoms' are:

1. New onset dyspepsia in individuals over age 50 years old
2. Dyspepsia associated with dysphagia and/or weight loss
3. Those with evidence of gastrointestinal bleeding (occult blood, anemia, hematemesis, and.or hematochezia/melena)
4. Those with signs or symptoms of UGI tract obstruction (e.g. early satiety, vomiting)
5. Those whose ethnic and/or racial background is associated with increased risk for upper gastro intestinal malignancies or other significant disease states.

The link between *H.pylori* infection and gastric cancer has been established through a series of epidemiological studies and has been corroborated by studies that support a strong biological plausibility for this relationship. The ultimate proof came from clinical observations and clinical and therapeutic trials which lead to the formulation of the statement in Maastricht III guidelines that *H.pylori* eradication has the potential to prevent cancer (Malfertheiner et al.,2007). The epidemiologic

evidence relating *H. pylori* infection with gastric cancer came initially from three nested case control studies which all showed that cancer patients had a higher *H. pylori* seroprevalence compared to controls and the risk associated with a positive serology varied between 2.1 and 8.7 (Forman et al.,1991; Parsonnet et al.,1991). Gastric cancer is one of the leading causes of cancer related deaths in the world. On a worldwide scale, crude gastric cancer rates are increasing (despite a decrease in age adjusted rates) because of increasing life worldwide. However, only cancers located distally to the cardia (non-cardia adenocarcinomas) are related to *H. pylori* infection; cancers located proximally are usually not.

A meta-analysis of 19 cohort studies and case-control studies published in 1998 estimated a summary odds ratio of 1.92 indicating an approximately 2-fold risk of gastric cancer among infected individuals (Huang et al.,1998). In addition to non-cardia adenocarcinoma, gastric B-cell lymphomas, which account for about 5% of all gastric malignancies, are also linked to a higher infection rate with *H. pylori* (Bayerdorffer et al.,1995) and eradication of *H. pylori* usually leads to regression of mucosa-associated lymphoid tissue lymphomas.

1.4 Diagnostic methods for detection of *Helicobacter pylori*

A large number of tests are now available for the diagnosis of *H.pylori* infection. In general, diagnostic testing for *Helicobacter pylori* can be divided into invasive and noninvasive techniques based upon the need for endoscopy. As mentioned earlier on, invasive method include gastric biopsy taken during upper

endoscopy that are tested for histobiochemistry, rapid urease test (RUT), culture and polymerase chain reaction and non invasive through serology (serum IgG), urea breath test(UBT), urinary excreation of (15N) ammonia and stools antigen assay.

There are still some unresolved issues in defining the gold standard for the the detection of *H.pylori* infection in various age groups, geographical areas and in persons with chronic infection. Culture of *H.pylori* organisms is still the gold standard for the diagnosis of *H.pylori* infection. Although it is very specific, it is not sensitive (Warren and Marshall, 1983). The choice of test depends upon issues such as cost, availability, clinical situation, population prevalence of infection, pretest probability of infection, and factors such as the use of proton pump inhibitors and antibiotics, which may influence certain test results.

1.4.1 **When to test**

There are a number of clinical circumstances in which testing for *H.pylori* is considered. Recommendations for diagnostic testing for *H.pylori* were first proposed by the National Institutes of Health (NIH) in 1994 (NIH, 1994). More recent guidelines were published in 2006 by the European Helicobacter Study Group (EHSG) (Malfertheiner et al.,2007) and in 2007 by the American College of Gastroenterology (ACG) (Chey and Wong 2007).

ACG recommendations — The ACG guidelines made the following conclusions:

- Testing for *H.pylori* should be performed only if the clinician plans to offer treatment for positive results.
- Testing is indicated in patients with active peptic ulcer disease, a past history of documented peptic ulcer or gastric MALT lymphoma.
- The test-and-treat strategy for *H.pylori* (ie, test and treat if positive) is a proven management strategy for patients with uninvestigated dyspepsia who are under the age of 55 years and have no "alarm features".
- Deciding which test to use in which situation relies heavily upon whether a patient requires evaluation with upper endoscopy and an understanding of the strengths, weaknesses, and costs of the individual test.

1.4.2 **Invasive test**

1.4.2.1 **Histopathologic Examination of Gastric Biopsy Specimens:**

Histochemical and Immunohistochemical Stains

Histopathologic examination can be helpful in making the primary diagnosis of *H.pylori* infection. It also provides additional information regarding the presence of inflammation and the detection of intestinal metaplasia and mucosa-associated lymphoid tissue (MALT). In addition to this, bacteria can be visualized in histologic preparation of gastric biopsy specimens stained with a variety of methods. *H.pylori* can be detected in routine hematoxylin and eosin-stained biopsy specimens. However, variations in the quality of the stain, the paucity of bacteria in some specimens, and the need for exceptional diligence on the part of observers make the hematoxylin and eosin stain a suboptimal choice for the specific task of detecting *H.pylori*. Numerous special stains are now available for the optimal detection of *H.pylori* (Genta et al.,

1994;El-zimaity et al., 1998). Several anti-*H.pylori* antibodies are commercially available for the immunohistochemical detection of *H.pylori* in paraffin-embedded biopsy specimens. This method requires considerable expertise, but its sensitivity and specificity are high and some laboratories use it for routine clinical diagnosis (Jonkers et al., 199; Marzio et al., 1998). Immunohistochemistry may be particularly useful for the detection of the coccoid forms of *H.pylori*, but there is no evidence that detection of coccoid forms has any clinical utility. Because local laboratory conditions and financial constraints often determine the choice of stain more than the individual histopathologist's preference, no universal recommendation is made as to what technique should be used. However, the revised Sydney System for the classification of gastritis states that the use of a special stain is strongly recommended, particularly when the hematocylin and eosin stain fails to reveal organisms in a biopsy specimen with chronic active inflammation. Thus, although many positive cases can be recognized in a good hematoxylin and eosin stain, careful examination of a special stain is deemed essential before declaring an inflamed biopsy specimen histologically negative for *H.pylori* (Dixon et al.,1996).

In situ hybridization may be used for the detection of *H.pylori* in paraffin-embedded section (Barret et al.,1997; Bashir et al.,1994). Although in situ hybridization may turn out to be the most specific and sensitive method for the visual detection of *H.pylori* in biopsy specimens, the high cost and difficulty of this procedure seem to make this an overly optimistic version, especially when biopsies from different regions of the stomach must be examined for each patient.

Data with endoscopic brush cytology to detect organisms are also encouraging (Narvaez et al., 1995). The sample obtained with brushing can be examined using standard Gram stain techniques or special staining if the results of the Gram stain are inconclusive. Sensitivity of 98 percent and specificity of 96 percent have been reported (Huang et al., 1996). One study found a sensitivity of 95 percent for brush cytology, 81 percent for histology, and 72 percent for rapid urease testing compared to brush cytology or histology as the reference standard (Mostaghni et al.,2008). This test should be considered in patients with bleeding disorders that make forceps biopsy undesirable, but is otherwise rarely used in clinical practice. Smears of gastric mucus and exfoliated epithelial cells may be prepared by using techniques similar to those used to obtain specimens for cytologic examination. Smears are usually stained with Gram stain, and allow the detection of bacteria within minutes of the endoscopic procedure (Dalla et al.,1996, Ghoussoub et al., 1997).

Histologic analysis is most often used as the gold standard for detection of *H.pylori* infection because in theory it is the most standardized procedure and provides an objective and permanent record of whether the bacteria are present or absence. The histopathologist has an additional advantage because, even when the bacteria are sparse, the other feature of *H.pylori* gastritis are usually evident.

The overall sensitivity of histophalogic examination varies from 80-95% depending upon the type of staining and immunocytochemical used to detect *H.pylori*. However the specificity of this test can achieved up to 96-98%.

Potential problems with histologic examination include:

- The density of *H.pylori* can vary at different sites, possibly leading to sampling error (Genta et al.,1994).
- Interobserver variability (Faigel et al., 1996).
- The sensitivity of histology may be decreased in patients taking antisecretory therapy, but is still higher in this setting than biopsy urease testing.
- Quality of the stainning

The accuracy of histologic diagnosis of *H.pylori* infection can be improved by using special stains such as Giemsa or specific immune stains (Wright et al., 2006).

1.4.2.2 **Bacterial Culture**

H.pylori is best cultured in a microaerophilic and humid atmosphere, usually 5% O_2^-, 10% CO_2^-, 80% to 85% N_2 99% to 100% humidify; and temperature between 33^0C. Incubators containing 10% to 15% CO_2 or anaerobic jars can also be used to culture *H.pylori*. Most culture media use fresh horse or sheep blood, and both selective media, containing antibiotics such as vancomycin, trimethoprim and amphocetirin B to suppress contaminants and nonselective media are used. The highest rate of success are reported from laboratories that prepare their own media. Colonies appear within 14 days, usually 3 to 7 days. It is considered imprudent to discard the plates before 14 days. Isolates are identified as *H.pylori* by Gram stain and biochemical identification based on positivity for urease, catalase and oxidase. Because many clinical facilities are not required to perform the time consuming procedures necessary to culture *H.pylori*, several methods for transportation have been revised (Hulst et al.,1996, Rautelin et al., 1997). *H.pylori* is sensitive to drying in

aerobic conditions. Special techniques are necessary for storage of biopsy specimens and clinical isolates. The most widely used method consists of freezing the fresh biopsy specimens in a glycerol-containing media such as skim, milk, brucella broth or cysteine-albini medium. Biopsy sample can be stored indefinitely at -70^0C and for up to a week at -20^0C. Pre immersion of the biopsy forceps in formalin does not reduce the yield of culture, so it is not crtitical to take biopsies for culture before those for rapid urease testing or histologic analysis.

The sensitivity of this method varies from 70-80% depending upon the preparation of the culture media and adequacy of the storage system of the culture media. Culture is theoretically the most specific with specificity up to 100%.

H.pylori is a fastidious microorganism that many laboratories find difficult to isolate. Culture also requires transport from the endoscopy laboratory to the microbiology laboratories. Delay, drying and poor choice of transport media all serve to reduce the value of culture as a clinically useful diagnostic test. Methods to transport and store biopsies before culture have been published but choice of culture media and culture condition as well as expertise and experience of the laboratory still influence the results of culture.

1.4.2.3 **Polymerase Chain Reaction**

Polymerase Chain Reaction (PCR) is a technique that allows the amplicifation of a deoxyribonucleic acid (DNA) template into multiple copies through sequential round of DNA replication by DNA polymerase. This permits the detection of a DNA fragment that can be resolved and visualised in agarose gels. A number of protocols have been devised but few have been properly standardized. The practical usefulness of PCR for the detection of *H.pylori* infection has been difficult to define because this

technique is much more sensitive than the other possible gold standards, such as the histopathologic detection of *H.pylori*. Thus, cases are frequently encountered in which no bacteria can be identified by histopathologic examination, yet the PCR yields a positive results (Nguen et al., 1995)

1.4.2.4 **Rapid Urease Test (RUT)**

Rapid Urease test is a fast, accurate and inexpensive method to diagnose *H.pylori* infection in the endoscopy suite. These tests are intended to detect urease enzyme in gastric mucosal biopsy specimens for determination of *H.pylori* in symptomatic patients. These assays exploit the high urease content of *H.pylori* (Laine et al., 1996; Yousfi et al., 1996). To perform the test, a fragment of gastric mucosa is placed into a medium containing urea and a pH indicator. Test for gastric urease are specific for *H.pylori* because mammalian cells normally do not produce urease and very few microorganisms survive in the stomach, except for *H.pylori*

H.pylori produces large amounts of urease enzyme. Although urease primarily allows *H.pylori* to utilize urea as a nitrogen source, the breakdown of urea also produces high local concentrations of ammonia, which enable the organism to tolerate low pH (see reaction below).

$$(NH_2)CO + 2H_2O + H^- \longrightarrow 2NH_4^- + HCO_3^-$$

Urea urease ammonium bicarbonate

The urease produced by *H.pylori* hydrolyzes the urea, releasing ammonia, which raises the pH of the broth or agar. An appropriate indicator (e.g. phenol red) changes color as the pH increases. In the first commercially produced RUTs, the

CLOtest (TriMED specialities, Inc., Lenexa, KS), contains original yellow urea gel capsule with chromatin index into which the specimen is placed becomes red within minutes to hours, depending on the quantity of bacteria present. Three tests are available commercially in the United States, but any laboratory can easily produce a successful medium. A homemade test can be made with a solution containing 2g urea, 10ml of 0.6% (w/v) phenol red, and 20 mg sodium oxide in 100ml of 0.01 mol/L sodium phosphate buffer, pH 6.5. A 0.5 ml dram vial is filled with 50Ul of this solution and biopsy specimens are added in the endoscopy room. The test is positive if the medium changes from orange to definite pink. Commercial tests using urea impregnated agar (hpfast, GI Supply and CLOtest, Camp Hil. PA) have similar chemical reaction principle but offer higher diagnostic accuracy. These tests uses a detergent to help release the urea and starts the reaction at a pH non-below *H.pylori* thus theoretically increasing the specificity.

Pronto Dry® is also one the commercial RUTs widely used around the world. Pronto Dry® consists of a dry filter paper containing urea, phenol red (a pH indicator), buffers and a bacteriostatic agent, in a sealed plastic side. If the urease enzyme of *H.pylori* is present in an inserted tissue sample, the resulting decomposition of urea causes the pH to rise and the color of the dot turns from yellow to a bright magenta. The tests should be stored at room temperature. Pronto Dry has a shelf life of 24 months.

If urease is present in the tissue, an expanding magenta color external ring will be noted around the biopsy specimens, or the Pronto Dry will gradually change to a

deep orange, then magenta color. A pink-magenta ring at 1 hour is a positive reaction. A negative result is when the external ring is still yellow 1 hour after insertion of the specimens. Subsequent color changes may occur, although in most cases a stable magenta or yellow color will be present. Pronto Dry test can diagnose *H.pylori* infection with 98% sensitivity, 97% specificity (Chomvarian et al., 2005; Chang et al.,2005; Said et al., 2004)

The overall sensitivity and specificity for these two RUTs is between 95% to 98%. The sensitivity of these commercial tests compared with histopathologic examination is extremely high, in most cases approaching 100%. This is supported by higher negative predictive value in a few series of studies (Yakoob et al.,2006, Chomvarin et al.,2005). Therefore, it is considered as preferrable method for test and treat with low false negative results. The speed of the reaction is related to the number of bacteria present. Thus, the most rapid results are obtained if several specimens taken at different gastric area or a specimen taken with large-cup forceps.

The advantages of Pronto Dry® compared to CLOtest are, it can be stored in the room temperature, ready to used (no need to warm the kit as oppose to CLOtest) and offering a sooner diagnosis (fast reaction). In addition, there was a linear correlation between the histology grading and Pronto graded chromatin index, regarding the stomach mucosal colonization density of *H.pylori* (John, 2007).