

# perspective

## Discrepancies in *H. pylori* Testing

*Customer Feedback Indicates Positive Results for H. pylori in Stool may not Always Correlate with Breath Hydrogen Testing or Serology*

In recent months a number of customers have raised concerns over discrepancies between our stool test for *H. pylori* and more conventional tests such as the urea hydrogen breath test (UBT) and serology antibody tests. Recent scientific literature suggests that such differences may largely be explained by the status of *H. pylori* infection.<sup>1-3</sup> Further to this, epidemiological data indicates that rates of *H. pylori* infection can be well over 50% in some regions of the world, which translates into a significant incidence in the average patient population.<sup>4</sup> Lastly, the tendency for *H. pylori* to elicit symptoms of dyspepsia in certain individuals, while being asymptomatic in others also adds to the complexity of discrepancies between molecular and standard tests for *H. pylori*.<sup>5-7</sup>

The ensuing article will describe in further detail the epidemiological data concerning *H. pylori* infection in Australia; document the studies which point to differences in status of *H. pylori* infection as an explanation for discrepancies in testing; and document the typical symptomatic profile of patients relative to the status of *H. pylori* infection. For specific information on discrepancies between PCR-based stool tests for *H. pylori* and the UBT, clinicians can read our previous article on this issue.

### **Prevalence of *H. pylori* Infection as an Explanation for Higher Incidence Using Molecular Detection Methods**

Recent statistics on the epidemiology of *H. pylori* quoted in a special 2009 issue of the journal *Helicobacter* show average prevalence rates ranging from under 10% in a sample of Japanese children to over 80% in a sample of African refugees living in Australia.<sup>4</sup> Variations in prevalence of *H. pylori* is known to be affected by age, region, country and socioeconomic status, with the infection rate of individuals in developing nations generally higher than in industrialized nations, most likely due to poor sanitary conditions. Some authors have gone so far as to argue that *H. pylori* is part of the normal flora in humans.<sup>8</sup>

#### *H. pylori* Prevalence in Australia

With regard to the Australian population, the prevalence of *H. pylori* infection is estimated to be between 15 and 38%.<sup>9-14</sup> Most studies have used *H. pylori* immunoglobulin G antibody status as the marker for *H. pylori* infection in random samples of the Australian adult population.<sup>9-14</sup> Many of the studies were conducted using either an Anglo-Celtic population or a population with >90% born in Australia and as a result may not represent the increasingly diverse racial background of current urban Australian society,<sup>13</sup> with approximately 30% of the population born overseas according to Australian Bureau of Statistics Census 2006.<sup>15</sup> Subgroups of the Australian population may have a higher incidence of *H. pylori* infection, such as immigrants or indigenous peoples.<sup>13</sup>

### *H. pylori* Prevalence in Indigenous Australians & Immigrants

Indeed, a study published this year in *The Pediatric Infectious Disease Journal*, found an overall prevalence of *H. pylori* of 44.2% in a sample of Australian Aboriginal children between 4 months and 2 years admitted to hospital with acute diarrheal disease from remote and rural communities across Northern Territory.<sup>16</sup> Similarly, as mentioned above, another study published this year showed an infection rate of 82% in a sample of 182 African refugee children (aged < 16 years) at their initial health assessment after resettlement in Australia.<sup>17</sup>

### *A Paradigm Change for H. pylori* Infection in Australia

Given the diverse racial background of current urban Australian society, the above studies suggest it is dangerous to extrapolate general incidence values of *H. pylori* from urban Australian populations to more regional and ethnically diverse Australian populations. What is clear however, from the large body of data concerning the epidemiology of *H. pylori* is that the organism is one of the most common pathogenic bacteria found in humans worldwide.<sup>4</sup> As such, it should not come as a surprise when *H. pylori* is detected in a patient's stool specimen. Perhaps, a change in paradigm is required with regard to the prevalence of *H. pylori* in the average Australian patient and its possible contribution to gut health/disease.

### **Density of *H. pylori* as an explanation for Discrepancies in Diagnostic Testing**

A recent study by researchers from Budapest, Hungary sought to determine the local densities of *H. pylori* in gastric biopsy specimens using real-time PCR-based methods and compare these with results of the urea breath test (UBT), serological testing and histology.<sup>1</sup> The researchers hoped to determine the differences in density of *H. pylori* biopsy specimens from patients with antral erosions secondary to *H. pylori* infection and how these compare with the results of other non-DNA-based diagnostic assays, namely, UBT, serological testing and histology.

The study population consisted of 53 *H. pylori*-positive patients. These patients were divided in three groups according to whether or not they had gastritis, intestinal metaplasia and/or intestinal atrophy. Of the 53 *H. pylori*-positive patients, 41 had complete antral erosions. None of the patients had previously received antibiotic treatment for *H. pylori*. Upper endoscopy was performed and three gastric biopsy specimens were taken from the erosions (if available), the antrum and the corpus.

#### *The Effect of Atrophic Gastritis on H. pylori* Density

PCR was shown to positively correlate with UBT, serology and histology and had the highest sensitivity and specificity of all methods. Interestingly, the researchers found significantly increased *H. pylori* density in the erosions when compared with the healthy part of the antrum and corpus. Another important finding was that in atrophic gastritis patients, the density of *H. pylori* in the antrum was significantly lower than in the corpus. This is in line with other research which points towards a significant decrease in sensitivity and specificity for histology, serology and culture when attempting to identify *H. pylori* infection among individuals with atrophic gastritis and/or intestinal metaplasia.<sup>23</sup>

#### *PCR Suited to Detection of Low Numbers of H. pylori*

The recent proliferation in development of PCR-based methods for detection of microorganisms in stool is in accordance with the significantly improved sensitivity and specificity associated with such methods. In their discussion the researchers from Budapest, Hungary refer specifically to the dramatic increase in the development and application of PCR-based *H. pylori* assays in recent years<sup>18, 19</sup> and cite the following advantages:

*“Owing to its high sensitivity the PCR method is suitable for diagnosis when an organism is present in low numbers, is slow growing, or is difficult to identify.”<sup>1</sup>*

#### *The Effect of H. pylori* Density & Strains on Sensitivity of UBT & Histology

Regarding the significant differences in sensitivities between the diagnostic tests for *H. pylori*, the authors from Budapest, Hungary offered the following explanation:

*“On the basis of our results, local density and number detected by PCR can be high enough so that *H. pylori* can contribute to epithelial cell layer destruction; however, the number of bacteria is too low to be detected by UBT, urease, or histology. The presented technique can be offered for the evaluation of cases with UBT results in the gray area and in cases where macroscopic erosion(s) exists after *H. pylori* eradication, determined by UBT.”<sup>1</sup>*

A similar study using real-time PCR to compare the accuracy of the UBT and histological grading for estimation of the density of *H. pylori* in gastric mucosa was undertaken by researchers from Tokyo, Japan in 2002.<sup>3</sup> Like the study above, the authors found a significant positive correlation between real-time PCR and histology, rapid

urease test, culture and urea breath test, with real-time PCR showing 100% specificity and sensitivity. However, regarding the false negative results from histological examinations, the rapid urease test and the UBT, the authors stated the following:

*"In this study, false negative results from histological examinations, the rapid urease test and the UBT were considered to indicate the lower sensitivity of these methods, because the density of H. pylori genomes in most of the patients with such false negative results was lower than the 25th percentile for patients with true positive results."<sup>3</sup>*

When discussing the specific drawbacks of the UBT, the authors mention:

*"Differences in H. pylori strains may account for differences in urease activity, so quantitative results of the UBT may be inaccurate. Furthermore, the corpus and fundus of the stomach may be coated unevenly with urea if the protocol of the UBT, including positioning of the patient, is not strictly followed."<sup>3</sup>*

Yet another study by researchers from Tehran, Iran sought to compare biopsy-based tests with stool-PCR in a sample of children admitted to a local medical centre for persistent upper gastrointestinal problems.<sup>2</sup> A very similar outcome was found to the studies described above, namely, an association between higher score of *H. pylori* in histology and a positive stool-PCR. In the words of the authors:

*"...the degree of stomach colonization by H. pylori may be important for successful detection of DNA in stool samples. Otherwise, the amount of bacteria excreted in stool may reveal information on the status of H. pylori infection."<sup>2</sup>*

### *Irregular H. pylori Stomach Distribution Explains Discrepancies in Histology Results*

Taken together, the above studies provide sound evidence that the human stomach can be covered by different numbers and genotypes of *H. pylori*, such that the mean density of *H. pylori* genomes in paired biopsy samples from the gastric antrum and corpus may not reflect total bacterial numbers of *H. pylori* in the stomach. This in turn provides a sound theory to explain differences between the results of PCR-based DNA diagnostic tests for *H. pylori* and histology.

### *UBT & Histology vs PCR*

UBT values in the gray area are likely to be associated with lower numbers of *H. pylori* in stool,<sup>1</sup> while discrepancies between histology and PCR-based tests are likely to be explained by sampling error associated with histological examination following endoscopy.<sup>1</sup> The accuracy of histology is also dependent on the stain selected and on the pathologist's skill.<sup>1</sup> The advantage with PCR-based stool testing is that regardless of the specific location of the gastric erosion, *H. pylori* will transit down the GI tract eventually ending up in the stool, thus alleviating issues associated with irregular urea production and uneven colonisation in the stomach.

### *Serology vs PCR*

Differences between PCR-based stool tests and serology are likely to be explained by the superior specificity and sensitivity of molecular methods versus serology for *H. pylori*, which has been widely documented.<sup>20</sup> The other documented drawbacks associated with serology-based *H. pylori* testing is the use of strains not specific to the particular geographical area in question<sup>21, 22</sup> and the fact that a positive result does not necessarily indicate a current infection.

## **Typical Clinical Presentation Associated with *H. pylori* Infection**

A common observation cited by practitioners to argue against the validity of a positive finding for *H. pylori* in stool is that the patient is asymptomatic. It is well documented however that infection with *H. pylori* can be asymptomatic in a significant portion of individuals.<sup>5-7</sup> A recent study by researchers from Varanasi, India, measured the prevalence of *H. pylori* in saliva and stool specimens from asymptomatic children and adults using nested PCR.<sup>5</sup> The authors reported a detection rate in stool of 4.25% in 5 y, 13.64% in 6-10 y, 50% in 11-16 y, 64% in 17-30 y, 58.62% in 31-45 y and 61.1% in 45-60 y of age groups, with very similar percentages in saliva.<sup>5</sup> Other studies published this year also found a significant incidence of *H. pylori* infection in asymptomatic children and adults using antibody tests in stool.<sup>6, 7</sup> Therefore, reliance on the presentation of clinical symptoms normally associated with *H. pylori* infection is not a justifiable means of predicting the presence or absence of *H. pylori* infection.

## **Summing Up**

The information in this article provides a rationale to explain discrepancies between *H. pylori* testing using the Metamatrix GIFx™ Profiles and more conventional tests such as UBT, serology and histology. In summary, *H. pylori* has a high prevalence in the general population, so regular presence of infection is to be expected.

Secondly, differences in regionality and density of colonisation by *H. pylori* in the stomach can explain discrepancies between PCR-based stool tests and UBT/serology. PCR-based detection has been shown to be significantly more sensitive and specific than serology thus explaining discrepancies between these tests. Lastly, clinical presentation of *H. pylori* infection is not uniform and thus cannot be used reliably as a means of determining likely infection.

We trust that the information within this article will provide clinicians the reassurance they need regarding decisions on the significance and treatment of *H. pylori* infection as detected using the Metamatrix GIFx™ Profiles. For any further questions relating to this article, please don't hesitate to contact Diagnostic Insight on 02 9966 9990; alternatively, questions can be emailed to info@diagnosticinsight.com.au.

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