



Helicobacter pylori

Sandra Wilkins, AS, HT(ASCP)
Michigan Institute of Urology

Helicobacter pylori (*H. pylori*), has infected up to one-half of the world's population. It is most common in developing countries where sanitation is poor, or in low socioeconomic areas where overcrowded living conditions are common. In many cases, the organism does not produce any symptoms and can go undetected for many years. This is especially true in children.

In 1875, German scientists documented helical shaped bacteria from the stomach lining. However, the bacteria could not be cultured and the findings were forgotten. *H. pylori* was rediscovered by Dr. Barry J. Marshall and Dr. J. Robin Warren of Perth, Western Australia nearly 25 years ago, in the stomachs of patients with gastritis & stomach ulcers. Previous to that time (1982/83), the conventional thinking was that **no** bacterium can live in the human stomach, as the stomach produced extensive amounts of acid which was similar in strength to the acid found in a car-battery. Marshall & Warren literally re-wrote the text-books with reference to what causes gastritis & gastric ulcers. In recognition of their very important discovery, they were awarded the 2005 Nobel Prize for Medicine & Physiology.

H. pylori, which was originally classified as *Campylobacter*, is a gram negative organism that has a smooth surface with multi-polar flagella, the long, slender projection from the cell body, and produces urease. Urease is an enzyme that aids in the breakdown of urea into carbon dioxide and ammonia. This organism is the leading cause of dyspepsia (heartburn, bloating and nausea), gastritis (an inflammation of the stomach) and gastric and duodenal ulcers. *H. pylori* is found in 60% - 80% cases of gastric ulcers. Increasing evidence also supports a link to gastric adenocarcinoma and gastric lymphoma.

The route of transmission is oral. *H. pylori* has been found in water, soil, contaminated food and utensils. The **fecal-oral** route is more prevalent in areas where sanitation is poor or where overcrowding is prevalent. *H. pylori* can be cultured from stools of most infected persons. People drinking contaminated water, or eating food washed with the tainted water, ingest the *H. pylori*. The other route is **oral-oral**. Those people infected with *H. pylori* often have gastro-esophageal reflux or belching. The bacteria rises to the mouth of the infected person, and can be transferred to another person through oral contact.

Survival of *H. pylori* in the Stomach

Once the organism is swallowed, the flagella attach themselves to the gastric or duodenal lining. The organism does not invade the tissue like many other organisms, but lies within the protective mucosal lining of the stomach. To aid in getting into the mucous layer, the *H. pylori* produces lipase and phospholipase, which are proteolytic of mucins. Inflammatory cells, sent by the body to kill the *H. pylori*, are unable to penetrate into the mucin layer. The same protective layer of mucus that protects the stomach from the gastric acid also protects and provides a home for the organism.

Within the mucosal layer, the organism is producing urease, which assists in its survival. The gastric juices and saliva (which ends up in the stomach when food is swallowed) contain a lot of urea. The *H. pylori* urease breaks down the urea into bicarbonate, carbon dioxide and ammonia. The bicarbonate and carbon dioxide are what cause the belching and reflux. The ammonia is used to neutralize the stomach acids, allowing the *H. pylori* to survive in the acidic stomach environment, when other bacteria cannot. *H. pylori* also produce protease and cytotoxins, which break down the protective mucin layer, exposing the gastric lining to damage by the stomach acid. At the same time, monophages and neutrophils are sent in to begin fighting off the infection, causing inflammation in the mucosal layer. These inflammatory cells, however, are not able to penetrate the mucin layer, where

the *H. pylori* are hiding. However, this process produces antibodies that are detectable in the blood serum. *H. pylori* also produces oxidases and catalases, to resist the oxidative killing by the monocytes and neutrophils. So not only can *H. pylori* hide from the inflammatory cells, they can also resist being destroyed by them. As a result, in chronic conditions, the body keeps sending in more and more inflammatory cells, none of which can eliminate the *H. pylori*.

The disease process, which begins with an infection and inflammation, leads to epithelial cell damage of the gastric mucosa. It is this cell damage that has been linked to gastric adenocarcinoma and gastric lymphoma.

Tests and Stains

There are some simple non-invasive testing methods. A urea breath test measures carbon dioxide levels after the ingestion of urea. Urea is ingested and is broken down by an enzyme produced by *H. pylori*. Carbon dioxide is produced and is exhaled and measured. Other more accurate methods are blood tests for serum antibodies. However, these tests do *not* indicate *where* the infection is, or how extensive it is. Nor do these non-invasive tests demonstrate the type of damage to the gastric lining. Remember, people can often be infected with *H. pylori* and yet have no symptoms or ulcers.

Invasive testing requires endoscopic examination where a biopsy is performed. Tissue samples can be used for histologic examination or a biopsy based rapid urease detection. The rapid urease detection requires a tissue sample to be placed in a treated gel. If the organism is present, it produces a color change in the gel because of the presence of urea.

Microscopic examination by H&E commonly reveals an inflammatory process of the mucosal lining. Special stains are required for the confirmation of the organism. Histologic methods range from Romanowsky, Giemsa, modified Giemsa, Genta, silver stains, and IHC to fluorescent DNA probes. Although the more advanced studies like IHC will yield a higher specificity, the cost and time is prohibitive. There are many staining procedures that are relatively quick and inexpensive.

Below are stains prepared for a NSH teleconference on Helicobacter pylori. There are several different staining protocols available, many available on the internet. Staining times can be modified to produce optimal results.

Giemsa –The metachromatic Giemsa solution is a combination of one or more types of eosin (e.g., eosin Y and/or eosin B), along with methylene blue which is a mixture of thionin blue dyes, which oxidize over time to several other blue/violet dyes (azures). The Giemsa solution is heated to 60 degrees Celsius, slides are added to the solution and are allowed to sit for 20 minutes. This can also be done at room temperature, and slides allowed to stain for 1-2 hours. This is a regressive stain. Differentiation is done with weak acid solution (to differentiate excess methylene blue), and then through graded alcohols (to remove excess eosin).

- ⇒ Results - Background varying shades of pink and pale blue; Microorganisms blue.
- ⇒ Advantages – Easy to do, reagents store for long time, can be made in lab or bought commercially, inexpensive.
- ⇒ Disadvantages – Easy to over or under differentiate.

Diff-Quik -A rapid method for quick diagnosis consists of commercially prepared stains. It is a variation of the Giemsa stain. Solution #1 – Methanol to fix the tissue, Solution #2 – an Acidophilic stain (eosin), Solution #3 - a Basophilic stain (methylene blue and azure II dyes), and a weak alcohol-acid differentiator.

- ⇒ Results – Background varying shades of pink/blue; Organisms are blue.
- ⇒ Advantages - Quick stain, commercially available, reusable reagents, very inexpensive.

⇒ Disadvantages – Easy to over or under differentiate. Background and mucin often similar shade of blue as the *H. pylori*, particularly if underdifferentiated.

Wright-Giemsa – this method was achieved by using the slide stainer in hematology that is used to stain blood smears. Steps in this protocol are a light eosin rinse followed by Azure B/methylene blue, Eosin and then methanol.

⇒ Results – Background is varying shades of blue/pink; Organisms stain blue.

⇒ Advantages – Hematology probably already has a stainer and protocol.

⇒ Disadvantages – Machine may be set up for alcohol- or air-dried fixed blood smears. Colors may not be as correct with formalin-fixed (or other fixed) tissue.

Toluidine Blue-Alcian Yellow (Leung & Gibbon's stain, Leung stain, or TBAY) This method consists of a solution of 1% periodic acid (used to oxidize the mucins in the stomach to aldehydes), HP Yellow or alcian yellow (dyes that stain the mucin aldehydes yellow), 10% acetic acid (used to adjust the pH of the yellow dye, making it more specific for the stomach mucins), and HP Blue or 1% toluidine blue (to stain nuclei and microorganisms).

⇒ Results – Background pale blue; Mucin yellow; Organisms blue.

⇒ Advantages – Stomach neutral mucins stain yellow, providing a location to look for *H. pylori* and a contrasting yellow background to the blue organisms.

⇒ Disadvantages – Alcian yellow is no longer being made.

Sayeed – this method consists of 0.5% periodic acid (used to oxidize the stomach mucin to aldehydes), Coleman's Feulgen (Schiff reagent, used to stain the oxidized mucin aldehydes pink), hematoxylin (to stain the nuclei blue) and methylene blue (to stain the microorganisms blue).

⇒ Results – Mucin pink; Nuclei blue; Organisms blue.

⇒ Advantages – Stomach neutral mucins stain pink, providing a location to look for *H. pylori* and a contrasting pink background to the blue organisms.

⇒ Disadvantages – Mucin can pick up a dark magenta color, obscuring the blue organisms.

Gimenez - This method uses a dilute buffered carbol fuchsin (Ziehl-Neelsen) for 1-2 minutes, followed by a water wash and stain with 1% malachite green for 45 seconds. Repeat the malachite green until section appears blue/green to the naked eye. Wash in water, blot and air dry. This is a really nice stain because the mucin stains a pale blue and the organisms really stand out. *Note: Fast green was substituted for malachite green in this photo.*

⇒ Results – Background blue/green; Organisms red/magenta.

⇒ Advantages – Fast stain, stock carbol fuchsin stable for months, inexpensive.

⇒ Disadvantages – Buffered working carbol fuchsin is stable for only 1-2 days. Getting correct shade of green background can be tricky.

Silver Stains – Dieterle, Warthin-Starry, Steiner & Steiner, each with some form of modification to the protocol. Common reagents among all: uranyl nitrate (sensitize the micro-organisms so they pick up silver faster than the background), silver nitrate (impregnates microorganisms), gum mastic or gelatin (slows down reducing of silver ions to black metallic silver, so tech can control the reaction), hydroquinone and/or formaldehyde (reducing chemicals that change silver ions to black metallic silver).

⇒ Results – Microorganisms brown-black; Background yellow-tan.

- ⇒ Advantages – microorganisms are black against a pale background. Microorganisms are larger in size, due to silver being deposited on top of bacteria. Also can see *H. pylori* on a lower power objective through the microscope.
- ⇒ Disadvantages – Expense of buying and disposing silver nitrate and/or uranyl nitrate. Easy to over- and under-develop. Silver precipitate sometimes occurs, mimicking and/or obscuring organisms.

Genta – This method is a Steiner & Steiner with counterstains of Alcian blue and H&E.

- ⇒ Results – Microorganisms brown-black; Intestinal or stomach dysplastic acid mucins light blue; Nuclei blue; Cytoplasm pink-tan.
- ⇒ Advantages – microorganisms are black and larger in size (see advantages of Silver Stains above). H&E allows histologic assessment (e.g., inflammation), Alcian blue demonstrates intestinal metaplasia (acid mucin).
- ⇒ Disadvantages – Expense of buying and disposing of silver nitrate and/or uranyl nitrate. Easy to over- and under-develop. Long turn-around time.

IHC – This method uses a detection, antibody, chromogen, counterstain system.

- ⇒ Advantages – Very sensitive and very specific.
- ⇒ Disadvantages – Expense, Time

Not everyone who has *H. pylori* will develop an ulcer. And not every ulcer is caused by *H. pylori* – stomach ulcers can also be caused by taking too many aspirin or other NSAIDs (non-steroidal anti-inflammatory drugs such as ibuprofen). It is therefore necessary to differentiate causes of ulcers, so that the patient can be properly treated by the clinician. This is the role of the Histotech and our arsenal of special stains for biopsies of the stomach and duodenum.

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AUTHOR: Sandra Wilkins, AS, HT(ASCP)

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1. All of the following enzymes are used by the *H. pylori* to survive in the stomach **EXCEPT**:
A. Esterase
B. Lipase
C. Protease
D. Urease
2. *H. pylori* is stained blue with which of the following procedures?
A. Gimenez
B. Leung
C. Sayeed
D. Steiner
3. Which of the following stains for *H. pylori* uses a counterstain specifically to demonstrate mucin cells?
A. Dieterle
B. Genta
C. Giemsa
D. Gram
4. TRUE or FALSE (circle one): *H. pylori* has been implicated in causing dyspepsia, gastritis, gastric ulcers, gastric adenocarcinoma and gastric lymphoma.

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