H. pylori testing

Disclosures

Co-founder and Chief Science Officer, TechLab

Learning Objectives

- Evaluate the appropriate testing methodology by balancing performance, economics, and workflow.
- Discuss the best patient care by providing accurate results for appropriate care within a test-treat-test framework, while also taking into account economic and convenience considerations for the patient.
- Examine the process of protecting the economic health and reputation of the institution while providing the best patient care.

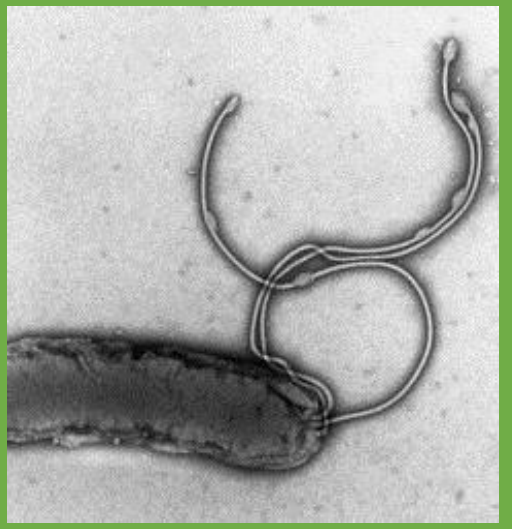
Agenda

- **Introduction and Background**
- **Diagnostic Tests for *H. pylori***
- **Treatment**

Introduction and Background

H. pylori: The Microbiology

- Gram-negative, helical-shaped
- Evolved to be highly specialized for its ability to grow in the stomach
- Virulence factors include 2-7 unipolar flagella and a potent urease
- The organism can evade the host immune response
- Marshall and Warren discovered *Campylobacter pyloridus* --- now known as *Helicobacter pylori*
- Slow growing (days) microaerophilic



Barry Marshall and Robin Warren

A complementary team that led to the discovery that gastritis and peptic ulcers were caused by the spiral-shaped organism now known as *H. pylori*

They were awarded the Nobel Prize for their discovery

H. pylori and *Campylobacter* species share some features

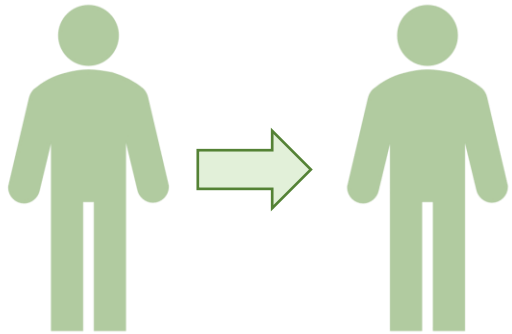
- Both are microaerophilic, spiral-shaped, and motile
- *H. pylori* targets the stomach mucosa and *Campylobacter* targets the intestinal mucosa
- They are recently identified pathogens --- *H. pylori* is human-specific and *Campylobacter* lives in avian/other animal hosts with humans as the bystander
- Both cause sequelae --- *H. pylori* can cause gastric cancer and *Campylobacter* is associated with certain neuromuscular diseases (e.g., Guillain-Barré Syndrome)

H. pylori is considered to be contagious and passed from person to person

Saliva

Fecal contamination (in food or water)

Poor hygiene practices



Peptic Ulcers

"Approximately 25 million Americans suffer from peptic ulcer disease at some point in their lifetime. Each year there are 500,000 to 850,000 new cases of peptic ulcer disease and more than one million ulcer-related hospitalizations."

90% of these are caused by *H. pylori*

Centers for Disease Control and Prevention. CDC twenty four seven. Saving Lives, Protecting People

Epidemiology

- *H. pylori* infection is considered a chronic condition
- How the organism is acquired is not always clear, but probably commonly infected during childhood
- Incidence and prevalence of *H. pylori* infection is higher among people outside North America
- Persons immigrating to U.S. have higher prevalence rates than persons born in U.S.
- Prevalence of 38% in a U.S. study using stool antigen testing
- Seropositivity rates outside North America exceed 70% but are much lower in Canada and the U.S.

Risk Factors

- Socially disadvantaged
- Infected parent
- Contaminated water

Major symptoms of *H. pylori* infection

- Diarrhea
- Peptic ulcers
- Severe abdominal pain
- Black tarry stools

Minor symptoms of *H. pylori* infection

- Belching
- Bloating
- Nausea/vomiting
- Abdominal discomfort

Who Should be Tested?

From the 2017 American College of Gastroenterology, those with:

- Active peptic ulcer disease
- Past history of peptic ulcer disease (unless cure was documented)
- Low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma
- History of endoscopic resection of early gastric cancer

Those who test positive should be offered treatment and tested for eradication of *H. pylori*

2017 ACG Guidelines have been extended

Gastroesophageal reflux disease, primarily those with dyspeptic symptoms or peptic ulcer disease

Persons on low-dose aspirin

Persons on non-steroidal anti-inflammatory drugs

Those who test positive should be offered treatment and tested for eradication of *H. pylori*

Diagnostic Tests for *H. pylori*

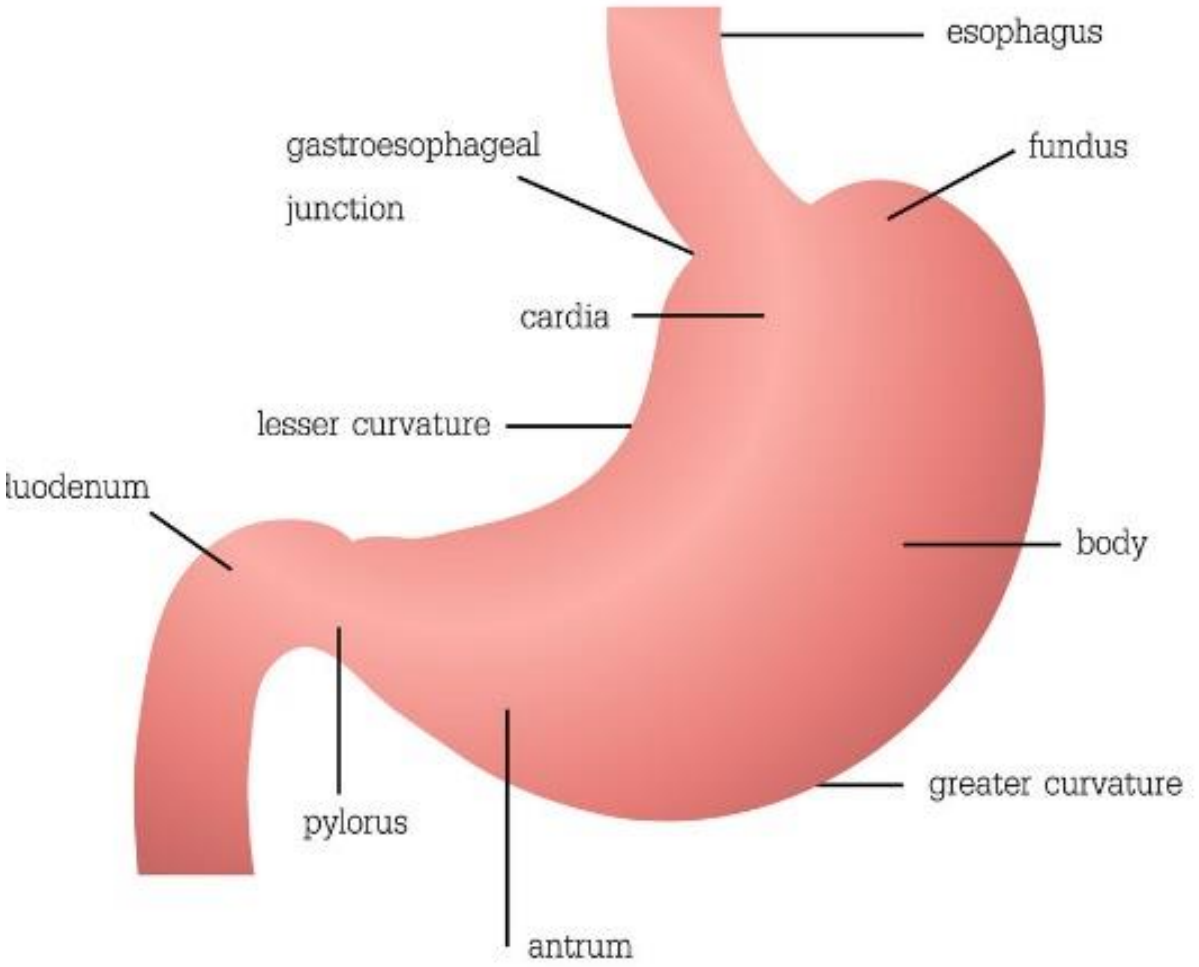
Diagnostic Tests for *H. pylori*

Invasive requiring biopsy:

- Culture
- Histology
- Rapid Urease

Noninvasive:

- Urea Breath Test
- Serology Test
- Stool Antigen



STOMACH ANATOMY

Endoscopy

Often used to diagnose *H. pylori*-associated diseases including:

Peptic ulcer disease (PUD) – a sore on the lining of the stomach, small intestine, or esophagus

Atrophic gastritis – chronic inflammation of the stomach, leading to the loss of glandular cells in the affected area

Mucosa-associated lymphoid tissue (MALT) lymphoma (originally stemming from B cells lining the tissue) --- most commonly seen in stomach

Gastric cancer

Gastric features of an *H. pylori* infection are very general

- Redness
- Mucosal swelling
- Changes in nodules
- Even with magnifying endoscopy, the specificity is insufficient (75%).

Histology for *H. pylori*

- Supporting studies show hematoxylin and eosin (H&E) staining identifies most cases
- Immunohistochemical stains may improve sensitivity and is highly specific but are more expensive
- Some studies suggest using immunohistochemistry in cases of unexplained gastritis where the organism load may be low
- In general, the method is limited by time and cost, experience, and variability

Smith et al. 2012. *Helicobacter pylori*: to stain or not to stain? Amer J Clin Pathol 137:733-738. https://doi.org/10.1309/AJCP8DGTAVG7MBMT

H. pylori culture from biopsy specimens

- Highly specific but sensitivity (50 to 90%) varies depending on the lab
- Critical factors for recovery of *H. pylori* include transport of biopsy samples, storage, media, and microaerophilic conditions
- To minimize overgrowth, selective media containing antimicrobial compounds can be used
- *H. pylori* can be recovered from stool samples, but recovery is low because of the other bacteria present

Miftahussurur, M., and Y. Yamaoka. Diagnostic methods of *Helicobacter pylori* infection for epidemiological studies: critical importance of indirect test validation. BioMed Res Internat 2016. http://dx.doi.org/10.1155/2016/4819423

H. pylori Culture from Biopsy Specimens

Grows slowly on agar --- several days at 37° C

Microaerophilic conditions are needed (5-10% O_2 is often used)

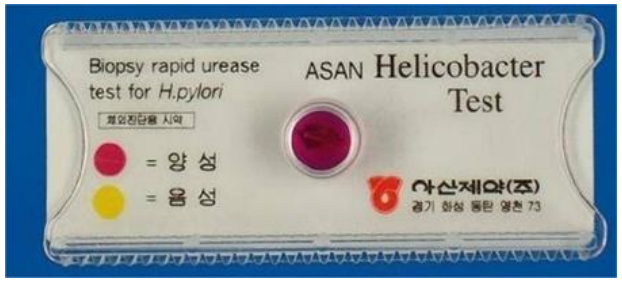
Blood agar will support growth of *H. pylori*

H. pylori on Columbia blood agar

H. pylori colonies on chocolate agar

Rapid Urease Test

- Also referred to as the CLO test (*Campylobacter*-like organism test)
- Rapid diagnostic test to aid in the diagnosis of infection by *H. pylori*
- Based on the ability of urease to convert urea to ammonia and carbon dioxide



Why *H. pylori* needs Urease

- *H. pylori* burrows into the mucosal layer in the stomach where the environment is less acidic
- The organism produces adhesins to help it adhere
- *H. pylori* produces larges amounts of urease (up to 5% of its protein)
- Urease breaks down urea from “gastric juice” to form ammonia and CO_2, both of which help to neutralize acids in the environment of the stomach
- Urease helps to keep the infection chronic, allowing *H. pylori* to survive in the stomach

Why urease is a marker for *H. pylori* infection

- Can be used to aid in diagnosis and to demonstrate eradication of *H. pylori*
- The patient must avoid proton pump inhibitors, bismuth-containing compounds, and antibiotics several weeks prior to testing to improve sensitivity
- There are other urease-producing organisms, but these don’t seem to be a major problem

How the Rapid Urease Test Works

Mucosal biopsy is taken from the stomach (typically the antrum)

The sample is placed into a medium containing urea and an indicator such as phenol red

Urease hydrolyzes the urea to ammonia, raising the pH of the medium

When the pH increases, the color changes from yellow (NEG) to red (POS)

Urea Breath Test for *H. pylori*

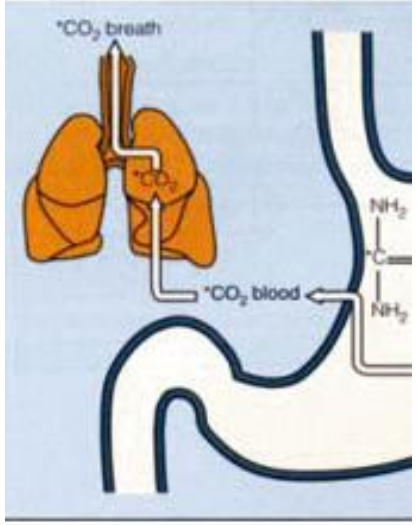
Patient drinks ^{13}C (non-radioactive) or ^{14}C (radioactive) urea

If *H. pylori* is present, urease breaks down urea into ammonia and CO_2

CO_2 travels to the lungs and is breathed out

A scintillation cocktail is used to determine the amount released with ^{14}C urea

Mass spectrometry is used for ^{13}C urea



Serology Testing for *H. pylori*

- Detection of antibodies to *H. pylori* is currently the most commonly ordered test
- Testing can be done in patients on PPIs, antibiotics, bismuth-containing compounds (Pepto-Bismol, Kaopectate). Tests are inexpensive and rapid.
- Limited clinical utility because of poor positive predictive value, especially in areas where the endemic rate is fairly low (e.g., in the U.S. where the rate is <30%)

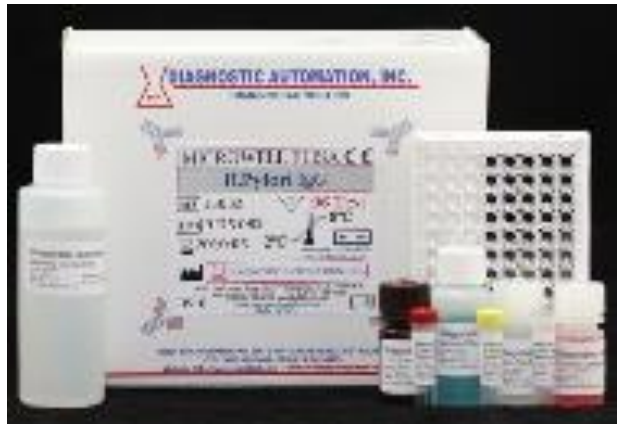
Serological Tests

There are many serology tests available around the world

The quality of capture antigen varies from test to test, resulting in significant performance differences

ELISA (microwell) and rapid dipstick formats are available

There are tests that claim to detect IgG, IgM, and IgA antibodies against *H. pylori;* serology tests that are FDA-cleared detect primarily IgG antibodies --- there is no indication that detecting specific classes of antibodies offers diagnostic advantages



Important Considerations for Serology Testing

- The 2017 ACG guidelines do not prefer serology
- Antibodies against *H. pylori* may persist for many years. A positive result for antibodies does not indicate an active infection and does not discriminate active and past infections
- Serology testing cannot be used to demonstrate eradication of *H. pylori* following treatment
- A negative result suggests the absence of *H. pylori*
- An increasing number of health insurers no longer reimburse for serology testing for *H. pylori*
- The Mayo Clinic discontinued offering this test in 2016

H. pylori Stool Antigen Tests

Rapid formats and ELISA microwell formats

Stool Antigen Tests

- Stool antigen tests are noninvasive and they have good sensitivity and specificity.
- Stool antigen tests are less expensive than the Urea Breath Test
- Stool antigen tests are more accurate than serology for determining active infection and are therefore preferred in the 2017 ACG guidelines
- Stool samples from persons with *H. pylori* infections will vary in consistency and will include solid, semi-solid, and diarrheal stools
- Stool antigen tests can be used in the initial testing and post-therapy to demonstrate eradication of *H. pylori*

Test Performance of Noninvasive *H. pylori* Stool Antigen Tests

Two Formats: 96-well and Rapid

Test	Sensitivity %	Specificity %
Premier HpSA Platinum® PLUS	96.1	95.7
ImmunoCard STAT!® HpSA	90.6	91.5
H. PYLORI CHEK™	100	96.1
H. PYLORI QUIK CHEK™	97.0	100

Test Performance of Noninvasive *H. pylori* assays

Quest Diagnostics Education Website and *H. pylori* review

Test	Sensitivity %	Specificity %	Diagnose Active Disease?	Confirm Eradication
H. pylori Stool Antigen (96-well)	96	96	Yes	Yes
H. pylori Stool Antigen (rapid)	91	92	Yes	Yes
Urea Breath Test	97	95	Yes	Yes
H. pylori Antibody (serology)	80 to 84	<80	No	No

Codes and Pricing

Test	CPT code	Reimbursement 2018
Histology	G0461	$74.16
Culture	87181	$5.86
Rapid urease test	87081	$9.97
Urea breath test	83013	$83.16
Serology	86677	$17.91
Stool Antigen	87338	$17.76

Treatment

Treatment

At least two antibiotics – typically clarithromycin paired with another antibiotic

Proton pump inhibitors - Omeprazole (Prilosec), Esomeprazole (Nexium), Lansoprazole (Prevacid) and Pantoprazole (Protonix)

Histamine (H-2) blockers that block histamine - Cimetidine (Tagamet) and Ranitidine (Zantac)

Bismuth subsalicylate – Pepto-Bismol and Kaopectate

Therapies for *H. pylori* infections

First line therapy examples:

- Clarithromycin triple containing clarithromycin, amoxicillin, PPI
- Bismuth quadruple consisting of bismuth subcitrate, tetracycline, metronidazole, PPI
- Salvage therapy should include antibiotics that were not used as a first line treatment

Multiple treatment strategies are described in: Chey et al. ACG Clinical Guideline: Treatment of *Helicobacter pylori* Infection. Amer J Gastroenterol 112:Feb 2017.

Antibiotic Resistance in *H. pylori*, U.S., 2009-2011

Antibiotic	Resistance rate
Levofloxacin	31%
Metronidazole	20%
Clarithromycin	16%

Shiota et al. Clin. Gastroenterol Hepatol 2015;13:16-1624

Gisbert et al. Aliment Pharmacol Ther 2012;35:209-221.

Antibiotic resistance testing for *H. pylori*

- Resistance testing can be done on isolates cultured from gastric biopsies
- Culture requires several days, can be challenging, and is affected by PPIs and antibiotics
- Resistance can be determined by agar dilution, disk diffusion and the E-test

Molecular analysis may be faster than resistance testing on isolated colonies

- *Current focus on mutations that lead to clarithromycin and levofloxacin resistance*
- Clarithromycin^{R} due to point mutations in 23S ribosomal subunit RNA
- Levofloxacin^{R} results from point mutations in the DNA gyrase subunit A
- These tests are not widely available in the U.S.

Should we test for treatment success after *H. pylori* eradication therapy?

Strong Recommendation from the 2017 ACG Clinical Guideline: "Whenever *H. pylori* infection is identified and treated, testing to prove eradication should be performed using a *urea breath test, fecal antigen test or biopsy-based testing* at least 4 weeks after the completion of antibiotic therapy and after PPI therapy has been withheld for 1-2 weeks."

A Test-Treat-Test approach is important. If treatment does not work, then the patient will continue to be infected with *H. pylori* and at-risk for *H. pylori*-related disease such as peptic ulcer disease and gastric cancer.

Test performance

- For biopsy sampling, histology and rapid urease outperform culture, but culture can be coupled with antibiotic susceptibility testing
- For noninvasive testing, the urea breath test and stool antigen test perform comparably; each provides better sensitivity and specificity than serology
- LIMITATION --- additional studies are needed to more accurately determine test performance values. These studies need to be performed in the U.S. where many, if not most, patients will be on PPIs and other medications versus those in other countries where PPIs may not be as widely used.

Summary

- Methods based on biopsy sampling include culture, histology/histopathology, and rapid urease test; these are commonly used as diagnostic aids and can yield additional information such as antibiotic resistance
- Methods that are not invasive are the urea breath test, serology, and stool antigen tests; the urea breath test and stool antigen test are the more accurate methods to determine an active infection
- Serology tests are not specific for an active infection
- Some studies support the use of multiple tests (e.g., histology combined with serology) for more accurate diagnosis

References

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