Test Definition: HPCRP

H pylori + Clarithro Resist PCR

Overview

Useful For

Assessing pure isolates of *Helicobacter pylori* to predict clarithromycin resistance or susceptibility

Highlights

This test detects the *Helicobacter pylori* 23S ribosomal RNA gene and the three most common 23S ribosomal RNA gene single nucleotide variations (A2143G, A2142G, and A2142C) leading to resistance to clarithromycin using viable or nonviable isolates to molecularly predict clarithromycin resistance or susceptibility.

Reflex Tests

Test ID	Reporting Name	Available Separately	Always Performed
PCRID	Identification by PCR	No, (Bill Only)	No

Testing Algorithm

See *Helicobacter pylori* Diagnostic Algorithm in Special Instructions.

When this test is ordered, the reflex test may be performed at an additional charge.

Special Instructions

- Helicobacter pylori Diagnostic Algorithm
- Infectious Specimen Shipping Guidelines

Method Name

Real-Time Polymerase Chain Reaction (PCR)

NY State Available

Yes

Specimen

Specimen Type

Varies

Advisory Information

This test uses isolates of *Helicobacter pylori* for testing. If testing directly from feces is desired, order HPFRP / *Helicobacter pylori* with Clarithromycin Resistance Prediction, Molecular Detection, PCR, Varies.

Additional Testing Requirements

1. If identification testing is needed; also order IDENT / Organism Referred for Identification, Aerobic Bacteria.

2. If susceptibility testing is needed; also order ZMMLS / Antimicrobial Susceptibility, Aerobic Bacteria, MIC, Varies.

Shipping Instructions

1. See Infectious Specimen Shipping Guidelines in Special Instructions for shipping information.

2. Place specimen in a large infectious container (T146) and label as an etiologic agent/infectious substance, if appropriate.

Necessary Information

Organism identification and specimen source are required.

Specimen Required

Supplies: Infectious Container, Large (T146)

Container/Tube: Agar slant or other appropriate media

Specimen Volume: Isolate

Collection Instructions:

1. Perform isolation of *Helicobacter pylori* in culture.

2. *H pylori* isolate must be submitted in pure culture. **Do not submit mixed cultures.**

Forms

If not ordering electronically, complete, print, and send a Gastroenterology and Hepatology Client Test Request (T728) with the specimen.

Specimen Minimum Volume

NA

Reject Due To

Agar plate; ESwab; Port-a-Cult	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Ambient (preferred)		
	Frozen		
	Refrigerated		

Clinical and Interpretive

Clinical Information

Helicobacter pylori is the main cause of peptic ulcer disease and, when left untreated, a risk factor for gastric cancer.Traditionally, *H pylori* diagnosis has included non-invasive tests (eg, urea breath test, fecal antigen test) or invasive tests (eg, gastric biopsy). Antimicrobial resistance in *H pylori* is poorly studied but is rising, challenging its treatment. In 2012, an international clinical consortium study group recommended monitoring of clarithromycin resistance rates and ceasing its use at a threshold range of 15% to 20%.(1) Local monitoring has been practically impossible as not all patients undergo invasive testing, which yields a culture isolate that can be subjected to susceptibility testing. Even if invasive testing is performed, the organism can be difficult to isolate in culture and is highly fastidious once isolated, oftentimes not being amenable to phenotypic susceptibility testing. Further, there are

only a handful of specialized clinical microbiology laboratories that perform *H pylori* susceptibility testing. In an internal study of local and referred isolates published in 2016, clarithromycin resistance was observed to be most commonly due to A2143G (70/88 isolates, 79.6%), followed by A2142G (12/88 isolates, 13.6%) and A2142C (3/88 isolates, 3.4%) alterations in the 23S ribosomal RNA gene.(2) Overall, one of these alterations was found in 97% of clarithromycin-resistant *H pylori* isolates studied.

Reference Values

Not applicable

Interpretation

Adetected result indicates the presence of *Helicobacter pylori* 23S ribosomal RNA gene; the presence or absence of the 3 most common 23S ribosomal RNA gene single nucleotide variations (A2143G, A2142G, and A2142C) is reported.

A not detected result indicates the absence of detectable *H pylori* DNA.

Cautions

This assay is used for testing of isolates of *Helicobacter pylori*. Request HPFRP / *Helicobacter pylori* with Clarithromycin Resistance Prediction, Molecular Detection, PCR, Feces if testing directly from feces is desired.

Potential cross-reactivity may occur with the following nonpylori *Helicobacter* species: *H acinonychis, H cetorum,* and *H mustalae* (not been reported to cause disease in humans) and *H canis, H cinaedi, H bizzozeronii,* and *H heilmannii* (infrequently found in humans).

This assay examines the 3 most common 23S ribosomal RNA point variants associated with clarithromycin resistance in *H pylori*. Other mechanisms of clarithromycin resistance are not assessed, nor are mechanisms of resistance to nonclarithromycin antimicrobial agents. The ZMMLS / Antimicrobial Susceptibility, Aerobic Bacteria, MIC, Varies assay is preferentially recommended for culture isolates to define a fuller spectrum of antimicrobial susceptibility (ie, to include antimicrobial agents beyond clarithromycin).

Supportive Data

During laboratory verification studies, 111 isolates of *Helicobacter pylori* (grown on Columbia agar with 5% sheep blood) with previous clarithromycin phenotypic susceptibility testing performed and which had undergone partial 23S ribosomal RNA gene sequencing were studied. This test matched phenotypic testing for 106/111 isolates (95.5% categorical agreement); a major error rate of 8.7% (2/23) and very major error rate of 3.4% (3/88) were observed. The assay results perfectly matched partial 23S ribosomal RNA gene sequencing data, including that performed on the 5 discordant isolates.

A subset of 45 of the isolates (including 1/5 isolates demonstrating a discordant result in the earlier study) were re-grown on tryptic soy agar with 5% sheep blood and re-assayed with this assay. The assay matched phenotypic testing in 44/45 isolates (97.8% categorical agreement); a major error rate of 0% and very major error rate of 3% (1/33) were observed. The PCR assay perfectly matched partial 23S ribosomal RNA gene sequencing data, including that performed on the single discordant isolates.

Clinical Reference

1. Malfertheiner P, Megraud F, Oâ€™Morain CA, et al: Management of *Helicobacter pylori* infection--the Maastricht IV/Florence Consensus Report. Gut 2012 May;61(5):646-664. doi: 10.1136/gutjnl-2012-302084

2. Chen D, Cunningham SA, Cole N, et al: Phenotypic and Molecular Antimicrobial Susceptibility of *Helicobacter pylori.* Antimicrob Agents Chemother 2017 Mar 24;61(4):e02530-16

3. Beckman E, Saracino I, Fiorini G, et al: A Novel Stool PCR Test for *Helicobacter pylori* May Predict Clarithromycin

Resistance and Eradication of Infection at a High Rate. J Clin Microbiol 2017 Aug;55:2400-2405

4. Monteiero L, Gras N, Vidal R, et al: Detection of *Helicobacter pylori* DNA in human feces by PCR: DNA stability and removal of inhibitors. J Microbiol Methods 2001 Jun;45(2):89-94

Performance

Method Description

Viable and nonviable clinical isolates are processed by transferring up to a 1 mcL loop full of isolate into a swab neutralization buffer (NB) tube for thermal/physical lysis and then diluted 1:100 prior to testing. The PCR assay employs a target-specific detection system including primers, as well TaqMan detection probes alongside a SimpleProbe for melt curve analysis-based genotyping targeting the 23S ribosomal RNA gene. The LightCycler 480 II instrument amplifies and monitors target nucleic acid sequences by fluorescence during PCR cycling. Detection of amplified product is based on the TaqMan probe principle. For PCR product detection, the TaqMan probe binds the complementary strand of amplified target. Specific PCR Taq polymerase with 5â€™-3â€™ exonuclease activity degrades the probe, releasing the fluorophore and breaking its proximity to the quencher molecule, allowing fluorescence of the fluorophores.. At the conclusion of PCR cycling, amplified product is thermally denatured and then cooled to allow for a fluorescein labeled SimpleProbe to anneal to an 18-base pair region of the amplified target that includes the 2 position mutations associated clarithromycin resistance. The temperature is slowly raised while consistently monitoring fluorescence. The process is completed in a closed system to mitigate contamination. Further, contamination control is achieved through UNG enzymatic treatment and a master mix which includes dUTPs.(Chen D, Cunningham SA, Cole N, et al: Phenotypic and Molecular Antimicrobial Susceptibility of *Helicobacter pylori* in the United States. Antimicrob Agents Chemother 2016;61:e02530-16)

PDF Report

No

Day(s) and Time(s) Test Performed

Monday through Friday

Analytic Time

3 days

Maximum Laboratory Time

4 days

Specimen Retention Time

30 days

Performing Laboratory Location

Rochester

Fees and Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification

Test Definition: HPCRP

H pylori + Clarithro Resist PCR

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

87150

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
HPCRP	H pylori + Clarithro Resist PCR	88509-5

Result ID	Test Result Name	Result LOINC Value
HPS2	Specimen Source	31208-2
HPORG	Organism Identified by Client	43409-2
608005	Helicobacter pylori Result	49101-9
608006	Clarithromycin Resistance Result	88509-5