Test Definition: HPFRP

H pylori + Clarithro Resist, PCR, F

Overview

Useful For

Aiding in the diagnosis of *Helicobacter pylori* infection and prediction of clarithromycin resistance or susceptibility directly from stool

Highlights

Detects the *Helicobacter pylori* 23S ribosomal RNA gene and the three most common 23S ribosomal RNA gene single nucleotide variations (A2143G, A2142G, and A2142C) that lead to resistance to clarithromycin

Testing Algorithm

See *Helicobacter pylori* Diagnostic Algorithm in Special Instructions.

Special Instructions

- Helicobacter pylori Diagnostic Algorithm

Method Name

Real-Time Polymerase Chain Reaction (PCR)

NY State Available

Yes

Specimen

Specimen Type

Fecal

Advisory Information

Confirmation of eradication testing should not be ordered until 4 or more weeks after cessation of treatment.

Necessary Information

Specimen source is required.

Specimen Required

The high sensitivity of amplification by polymerase chain reaction (PCR) requires the specimen to be processed in an environment in which contamination of the specimen by *Helicobacter pylori* DNA is unlikely.

Patient Preparation: Proton pump inhibitors, histamine H2preceptor antagonists and other antacids, as well as antibiotics and bismuth compounds, should be discontinued at least 2 weeks prior to testing.

Supplies: C and S Vial (T058)

Specimen Type: Preserved feces

Submission Container/Tube: Commercially available transport system specific for recovery of enteric pathogens from fecal specimens (15 mL of nonnutritive transport medium containing phenol red as a pH indicator, either Cary-Blair or Para-Pak C and S)

Specimen Volume: 5 mL

Collection Instructions:

1. Collect fresh fecal specimen and submit 1 gram or 5 mL in container with transport medium.

2. Place feces in preservative within 2 hours of collection.

3. Place vial in a sealed plastic bag.

Specimen Minimum Volume

1 mL

Reject Due To

Fecal swab ESwab transport medium Fecal ESwab transport medium Feces in gel transport medium ECOFIX preservative formalin PVA fixative	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Fecal	Refrigerated (preferred)	7 days	
	Frozen	7 days	

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 it's 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 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; also indicated is whether or

not one the 3 most common 23S ribosomal RNA gene single nucleotide variations (A2143G, A2142G, and A2142C) associated with clarithromycin resistance is detected.

A not detected result for *H pylori* indicates the absence of detectable *H pylori* DNA, but does not negate the presence of the organism and may occur due toinhibition of the polymerase chain reaction (PCR),sequence variability underlying primers or probes, or the presence of *H pylori* DNA in quantities less than the limit of detection of the assay.

Cautions

Avoidance of bismuth compounds, antibiotics, and acid suppressive drugs (histamine H2preceptor antagonists and proton pump inhibitors) should occur for at least 2 weeks before the test, as these medications may lead to a false-negative test result due to potential activity against *Helicobacter pylori*.

Test results should be used as an aid in the diagnosis. The single assay should not be used as the only criterion to form a treatment decision; results of this test should be correlated with clinical presentation and results of other laboratory tests. A negative result does not negate the presence of the organism or active disease.

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

This assay examines the three most common 23S ribosomal RNA single point variants associated with clarithromycin resistance. Other mechanisms of clarithromycin resistance are not assessed, nor are mechanisms of resistance to non-clarithromycin antimicrobial agents.

Supportive Data

During laboratory verification studies, 745 fecal samples previously tested with the Meridian Premier Platinum HpSA Plus fecal antigen test were assayed with this test. The assay detected *Helicobacter pylori* DNA in 306/335 antigen positive fecal samples (91% sensitivity [87.5-93.9%, 95% CI]). The *Helicobacter pylori* with Clarithromycin Resistance Prediction (HPFRP) assay also detected *H pylori* DNA in 12/410 antigen negative fecal samples (97.1% specificity [94.9%-98.5%, 95% CI]). Positive and negative predictive values were 96.2% (93.5-98.0%, 95% CI) and 93.0% (90.1-95.2%, 95% CI), respectively. Simple Kappa Coefficient measurement of the performance of the assay against that of the antigen test was 0.89 (0.85-0.92, 95%CI), an almost perfect correlation.(Landis JR, Koch GG, Biometrics 1977;33:159-174)

Assessment of clarithromycin resistance prediction was made by performing bidirectional Sanger sequencing on all HPFRP positive samples. All 76 samples with predicted clarithromycin susceptible *H pylori*, demonstrated wild-type 23S ribosomal RNA gene sequence at positions 2142 and 2143. All 37 samples with predicted clarithromycin resistant, *pylori* demonstrated single nucleotide polymorphisms of A2143G, A2142G, or A2142C in the detected *H pylori* 23S ribosomal RNA gene.

Clinical Reference

1. Malfertheiner P, Megraud F, O'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

Fecal samples (approximately 50-100 mg) are placed into 50% S.T.A.R. buffer and extracted on a Hamilton Microlab STAR device using a Mayo Microlab Maxwell HT Fecal DNA Purification Kit. The polymerase chain reaction (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 5'-3' exonuclease activity degrades the probe, releasing the fluorophore and breaking the 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 2 positions mutations at which are known to confer 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

Specimen Retention Time

7 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

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

87798 x 2

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
HPFRP	H pylori + Clarithro Resist, PCR, F	88509-5

Result ID	Test Result Name	Result LOINC Value
HPSRC	Specimen Source	31208-2
608002	Helicobacter pylori Result	91061-2
608003	Clarithromycin Resistance Result	88509-5