MAYO CLINIC LABORATORIES

# Test Definition: HPRP

Helicobacter pylori with Clarithromycin Resistance Prediction, Molecular Detection, PCR, Tissue

## Overview

### Useful For

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

### Reflex Tests

| Test Id | Reporting Name | Available Separately | Always Performed |
|---|---|---|---|
| TISSR | Tissue Processing | No | No |

### Testing Algorithm

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

For more information see *Helicobacter pylori* Diagnostic Algorithm.

### Special Instructions

- Helicobacter pylori Diagnostic Algorithm

### Highlights

This test detects the *Helicobacter pylori* 23S ribosomal RNA gene and the three most common 23S ribosomal RNA gene mutations (A2143G, A2142G, and A2142C) associated with resistance to clarithromycin.

### Method Name

Real-Time Polymerase Chain Reaction (PCR)

### NY State Available

Yes

## Specimen

### Specimen Type

Varies

### Ordering Guidance

If testing directly from feces is desired, order HPFRP / *Helicobacter pylori* with Clarithromycin Resistance Prediction, Molecular Detection, PCR, Feces.

For more information see *Helicobacter pylori* Diagnostic Algorithm.

## Specimen Required

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

**Submit only 1 of the following specimens:**

**Specimen Type:** Fresh tissue or biopsy
**Sources:** Stomach (or duodenum)
**Container/Tube:** Sterile container
**Specimen Volume:** Entire collection or 5 mm (3) approximate size of a pencil eraser
**Collection Instructions:**
1. Collect fresh tissue specimen.
2. Submit tissue in a sterile container (without adding anything).
3. Refrigerate or freeze the specimen.
**Specimen Stability Information:** Refrigerated (preferred) 7 days/Frozen 7 days

**Preferred:**
**Supplies:** Tissue Block Container (T553)
**Specimen Type:** Formalin-fixed, paraffin-embedded (FFPE) tissue block
**Sources:** Stomach (or duodenum)
**Container/Tube:** Tissue block
**Collection Instructions:** Submit FFPE tissue block to be cut and returned.
**Specimen Stability Information:** Ambient (preferred)/Refrigerated

**Acceptable:**
**Specimen Type:** Formalin-fixed, paraffin-embedded (FFPE) tissue scroll
**Sources:** Stomach (or duodenum)
**Container/Tube:** Sterile container for each individual cut section (scroll)
**Collection Instructions:** Perform microtomy and prepare five separate 10-micron sections. **Each section (scroll) must be placed in a separate sterile container for submission.**
**Specimen Stability Information:** Ambient (preferred)/Refrigerated

## Forms

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

## Specimen Minimum Volume

Fresh tissue or biopsy: 5 mm(3)
Formalin-fixed paraffin-embedded tissue block: One block
Formalin-fixed paraffin-embedded tissue scroll: Two 10-micron sections

## Reject Due To

| | |
|---|---|
| Tissue in formalin formaldehyde, or acetone FFPE slides | Reject |

### Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---|---|---|---|
| Varies | Varies | | |

## Clinical & Interpretive

### Clinical Information

*Helicobacter pylori* is a cause of peptic ulcer disease and, when left untreated, a risk factor for gastric cancer. *H pylori* diagnosis includes noninvasive tests (eg, stool polymerase chain reaction [PCR], urea breath test, stool antigen test) and tests requiring endoscopy to collect specimens for analysis. Several tests can be performed on gastric specimens, including *H pylori* PCR.

Antimicrobial resistance in *H pylori* is poorly studied but is rising, challenging its treatment. Assessment of antimicrobial resistance can guide treatment. Endoscopically collected gastric specimens can be cultured for *H pylori* and the recovered organism tested for phenotypic antimicrobial susceptibility. However, the organism can be difficult to isolate in culture, and even when isolated, may not amenable to phenotypic susceptibility testing due to its fastidious nature.

Clarithromycin resistance is most often associated with 23S ribosomal RNA (rRNA) gene mutations (particularly A2143G, A2142G/C). A systematic review and meta-analysis showed the sensitivity and specificity of detection of the *H pylori* A2142G/C and/or A2143G combination for prediction of clarithromycin resistance in *H pylori* in biopsy samples to be 96% each.

This test detects *H pylori* in gastric and duodenal biopsy specimens and, when detected, assesses for *H pylori* 23S rRNA gene mutations associated with clarithromycin resistance.

### Reference Values

Not detected

### Interpretation

A ‘detected’ result indicates the presence of *Helicobacter pylori* 23S ribosomal RNA gene; also indicated is whether or not one the three most common 23S ribosomal RNA gene mutations (A2143G, A2142G/C) 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 to inhibition 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

Testing should be performed at least 4 weeks after completion of antibiotic (including bismuth) therapy and after proton pump inhibitor (PPI) or vonoprazan therapy has been withheld for 2 weeks. False-negative results may occur if testing occurs prior to these recommended timeframes. Histamine 2-receptor antagonists have been shown to slightly decrease the sensitivity of some *Helicobacter pylori* tests and, if possible, should be discontinued 2 weeks before testing. Antacids do not appear to impair test performance and may be taken until one day before testing.

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 non-*pylori 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 mutations 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, limit of detection was established for all four targets (wild type, and clarithromycin conferring point mutations; A2143G, A2142G, A2142C) at single genomes/µl in both fresh tissue and formalin-fixed, paraffin-embedded (FFPE). Positive percent agreement was assessed using 56 FFPE gastric tissues known to contain *Helicobacter pylori*. The assay detected *H pylori* DNA in 53/56 (95% agreement) samples, with 18 samples predicted to be resistant to clarithromycin. Bi-directional Sanger sequencing confirmed the presence of mutations associated resistance to clarithromycin in all 18 samples, while all other samples displayed wild type. Negative percent agreement was assessed using 98 FFPE gastric tissues not known to contain *H pylori*. The assay did not detect *H pylori* DNA in any of the negative samples (100% agreement).

## 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;61(5):646-664. doi:10.1136/gutjnl-2012-302084
2. Chen D, Cunningham SA, Cole N, Kohner PC, Mandrekar JN, Patel R. Phenotypic and molecular antimicrobial susceptibility of Helicobacter pylori. Antimicrob Agents Chemother. 2017;61(4):e02530-16. doi:10.1128/AAC.02530-16
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;55(8):2400-2405
4. Marrero Rolon R, Cunningham SA, Mandrekar JN, Polo ET, Patel R: Clinical evaluation of a real-time PCR assay for simultaneous detection of *Helicobacter pylori* and genotypic markers of clarithromycin resistance directly from stool. J Clin Microbiol. 2021;59(5):e03040-20. doi:10.1128/JCM.03040-20
5. Savarino V, Tracci D, Dulbecco P, et al. Negative effect of ranitidine on the results of urea breath test for the diagnosis of *Helicobacter pylori*. AM J Gastroenterol. 2001;96(2):348-52. doi:10.1111/j.1572-0241.2001.03517.x

6. Chey WD, Grigorios L, Howden CW, Moss SF. ACG Clinical Guideline: Treatment of *Helicobacter pylori* infection. Am J Gastroenterol. 2017;112(2):p 212-239. doi:10.1038/ajg.2016.563
7. Jones NL, Koletzko S, Goodman K, et al. Joint ESPGHAN/NASPGHAN Guidelines for the Management of *Helicobacter pylori* in Children and Adolescents. J Pediatr Gastroenterol Nutr. 2017:64(6):991-1003. doi:10.1097/MPG.0000000000001594
8. Malfertheiner P, Megraud F, O'Morain CA, et al. Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. Gut. 2017;66(1):6-30. doi:10.1136/gutjnl-2016-312288

## Performance

### Method Description

Fresh tissue samples (approximately 2 x 2 x 5 mm) are placed in a polymerase chain reaction (PCR) Bead Tube containing Tris-EDTA buffer, and proteinase K and digested on the Bertin Precellys Disruptor. Formalin-fixed, paraffin-embedded (FFPE) tissue scrolls are placed in a PCR bead tube containing Tris-EDTA buffer and proteinase K and undergo a prolonged heat treatment for digestion. Once digested, samples undergo DNA extraction on a MagNA Pure 96 instrument. 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 is used to amplify and monitor 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 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. Contamination control is enhanced using uracil -DNA glycosylase enzymatic treatment and a master mix which includes dUTPs.(Chen D, Cunningham SA, Cole NC, Kohner PC, Mandrekar JN, Patel R. Phenotypic and molecular antimicrobial susceptibility of *Helicobacter pylori*. Antimicrob Agents Chemother. 2017;61(4):e02530-16. doi: 10.1128/AAC.02530-16)

### PDF Report

No

### Day(s) Performed

Monday through Friday

### Report Available

4 to 6 days

### Specimen Retention Time

7 days

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Helicobacter pylori with Clarithromycin Resistance Prediction, Molecular Detection, PCR, Tissue

**Performing Laboratory Location**

Mayo Clinic Laboratories - Rochester Main Campus

## Fees & 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 account representative. 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. It has not been cleared or approved by the US Food and Drug Administration.

### CPT Code Information

87513

### LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| HPRP | H pylori + Clarithro Resist, PCR | 88509-5 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| HPS3 | Specimen Source | 31208-2 |
| 616027 | Helicobacter pylori Result | 91060-4 |
| 616028 | Clarithromycin Resistance Result | 88509-5 |