JAN 11, 2020

OPEN ACCESS

DOI:
[dx.doi.org/10.17504/protocols.io.54jg8un](https://doi.org/10.17504/protocols.io.54jg8un)

External link:
<https://doi.org/10.1371/journal.pone.0227275>

Protocol Citation: Eliana Rocio Rodríguez Gómez, William Otero Regino, Pedro A. Monterrey, Alba Alicia Trespalacios Rangel 2020. Molecular biology test for rapid detection *cagA* gene EPIYA motif in *H. pylori* isolates. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.54jg8un>

MANUSCRIPT CITATION: Gómez ERR, Regino WO, Monterrey PA, Rangel AAT (2020) *cagA* gene EPIYA motif genetic characterization from Colombian *Helicobacter pylori* isolates: Standardization of a molecular test for rapid clinical laboratory detection. PLoS ONE 15(1): e0227275. doi: [10.1371/journal.pone.0227275](https://doi.org/10.1371/journal.pone.0227275)

Molecular biology test for rapid detection *cagA* gene EPIYA motif in *H. pylori* isolates

PLOS One

Eliana Rocio Rodríguez Gómez¹, William Otero Regino², Pedro A. Monterrey³, Alba Alicia Trespalacios Rangel¹

¹Microbiology Department, Pontificia Universidad Javeriana, Bogotá, Colombia;

²Gastroenterology Unit, Clínica Fundadores, Bogotá, Colombia;

³Natural Sciences and Mathematics Faculty, Universidad del Rosario, Bogotá, Colombia.

Eliana Rodriguez

ABSTRACT

In this study , we aimed to determine the current circulation of *cagA* gene EPIYA motifs present in Colombian *Helicobacter pylori* isolates using a rapid molecular test. The *cagA* gene 3' region was amplified through conventional Polymerase Chain Reaction (PCR) and PCR products obtained were sequenced and analyzed with bioinformatics tools. Additionally, to confirm the prediction of the number EPIYA C repeats based on the PCR product molecular weight, reamplification and sequencing analysis were performed.

MATERIALS

MATERIALS

- GoTaq Green Master Mix **Promega Catalog #M7122**
- Wizard SV Gel and PCR Clean-Up System **Promega Catalog #A9281**
- primers **Contributed by users**
- Ultrapure(TM) Agarose **Thermo Fisher Scientific Catalog #16500100**
- 8-strip PCR tubes, emulsion safe (!) (e.g. TempAssure PCR 8-strips) **USA Scientific**
- SyberSafe DNA Gel Stain **Invitrogen - Thermo Fisher Catalog #S33101**
- 1x TBE buffer **Contributed by users**
- UltraPure™ DNase/RNase-Free Distilled Water **Thermo Fisher Scientific Catalog #10977015**

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

Created: Aug 02, 2019

Last Modified: Jan 11, 2020

PROTOCOL integer ID:
26475

Keywords: Amplification of *cagA* gene 3' region , reamplification and secuencing analysis.

1 The entire 3' repeat regions of the *cagA* gene was amplified by polymerase chain reaction (PCR) using the *cagA* primers, sense primer (CAGTF 5'-ACCCTAGTCGGTAATGGG-3') and antisense primer (CAGTR 5' GCTTTAGCTTCTGAYACYGC 3')

1.1 PCR amplification was carried out a volume of 50 μ L containing 0.3 μ M concentrations of primers (Invitrogen, Carlsbad, CA, USA), 100 ng *H. pylori* genomic DNA and 1 U of master mix for *Taq* DNA polymerase (Promega, Madison, USA)

1.2 The polymerase chain reaction conditions included:

Initial denaturation	95°C/ 10 min
Denaturation	95°C/ 30 sec,
Hybridization	52.3°C/30 sec

Exten sion	72°C/ 36 sec
Final exten sion	72°C/ 5 min

39 cycles

PCR amplification was carried out in a Thermal cycler T100 (Biorad, Hercules, CA, USA).

- 1.3 PCR products were analyzed by 2% agarose gel electrophoresis with Syber Safe and analyzed in the Gel Doc^{XR+} system (Biorad, Hércules, CA, USA).
- 1.4 Samples which presented two or more bands in agarose gels from PCR amplifications (samples with mixed infection), were visualized and cut using an UV transilluminator. Each gel band with different size was put in an eppendorf tube and then purified using the Wizard SV Gel and PCR Clean- Up System Kit (Promega, Madison, USA), according to the manufacturer's instructions. DNA concentration was determined by spectrophotometry using NanoDrop 2000 (Thermo Scientific, Wilmington, NC).
- 2 The DNA amplification obtained from gel bands was subsequently amplified by conventional PCR. Sequencing for PCR products and bioinformatic analysis.
 - 2.1 All PCR products from this study were sequenced by Universidad de Los Andes, Bogotá, Colombia. Sequencing reactions were carried out for both DNA strands (forward and reverse sense) by the Sanger method using as control for sequences the *H. pylori* reference strain (NCTC 11637)
- 3 Nucleotide sequences were aligned and analyzed using BLASTx, including *H. pylori* reference strain *cagA* sequences (NCTC 11637) (GenBank access AF202973). The deduced peptide sequences containing EPIYA motifs were aligned by CLUSTAL W (European Bioinformatics Institute <http://www.ebi.ac.uk/Tools/clustalw2/>).