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

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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.Aditionally, to confirm the prediction of the number EPIYA C repeats based on the PCR product molecular weight, reamplification and secuencing analysis were performed.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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MATERIALS

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

1 The entire 3´ repeat regions of the c*agA* 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 µl** containing 0.3 µM 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
Extension	72°C/ 36 sec
Final extension	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/).