



Helicobacter pylori

REF: CL20K

Detection of Helicobacter pylori by FAST PCR of a fragment of species specific antigen region

INTRODUCTION AND PURPOSE OF USE

The Helicobacter pylori system is a qualitative test that allows the DNA amplification, by means of FAST PCR, of species specific antigen region of Helicobacter pylori genome correctly extracted by biological samples. The amplification product is a 303 bp fragment and is detected by electrophoresis on (pre-cast) PCR CheckIT gel containing ethidium bromide.

CONTENT

The kit contains enough reagents to perform 48 amplification tests

Quantity	Description
R1 2 x 420 µl	Helicobacter pylori FAST PCR master mix (store at -20°C) Helicobacter pylori upstream primer, Helicobacter pylori downstream primer, dNTPs, Tris-HCl, KCl, MgCl ₂ , Fast Taq Polymerase, Nuclease-free water
R2 1 x 30 µl	Positive control Cloned DNA corresponding to the species specific antigen region
R3 1 x 30 µl	Negative control
R4 1 x 250 µl	Loading dye Gel-loading solution
R5 1 x 60 µl	100 bp ladder Containing 10 bands, with dimensions included between 100 bp and 1000 bp; the distance between two adjacent bands is 100 bp
R6 1 x 30 ml	TAE Tris-Acetate-EDTA buffer for electrophoresis run (concentrate)
5	Gels PCR CheckIT pre-cast with EtBr

MATERIALS AND STRUMENTATION REQUIRED BUT NOT SUPPLIED

Disposable latex powder-free gloves or similar material; Bench microcentrifuge (12,000 - 14,000 rpm); Micropipettes and Sterile tips with aerosol filter; Thermal cyclers; Horizontal electrophoresis unit and power supply for electrophoresis; U.V. transilluminator and camera for gel recording.

ACCESSORY PRODUCTS

EX15 – DNA extraction from biopsy

The kit allows the DNA extraction from Human samples. The kit contains enough reagents to perform the DNA extraction from 50 samples.

CL54K - β-Globin

Control system of DNA extraction through the genomic amplification of β-Globin human gene.

SAMPLES AND STORAGE

The Helicobacter pylori system must be used with extracted DNA from biopsy. The samples collected must be shipped and stored at +2 - +8°C and used within 3 days from the collected data. Store the sample at -20°C if used after 3 days.

PRECAUTIONS USE

This kit is for in vitro diagnostic (IVD), for professional use only and not for in vivo use.

Keep the Fast PCR Master Mix on ice while in use and return to -20°C immediately after the use.

At all times follow Good Laboratory Practice (GLP) guidelines.

Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.

Avoid any contact between hands and eyes or nose during specimens collection and testing.

Handle and dispose of all used materials into appropriate bio-hazard waste containers. It should be discarded according to local law.

Keep separated the extraction, the reagents preparation and products detection area.

Never pipette solutions by mouth.

Amplification products must be handled in such a way to reduce dispersion into the environments as much as possible, in order to avoid the possibility of contamination.

Avoid the air bubbles during the master mix dispensing. Eliminate them before starting amplification.

Wash hands carefully after handling samples and reagents. Do not mix reagents from different lots.

It is not infectious and hazardous for the health (see Material Safety data Sheet – MSDS).

Do not eat, drink or smoke in the area where specimens and kit reagents are handled.

Read carefully the instructions notice before using this test. Do not use beyond the expiration date which appears on the package label.

Do not use a test from a damaged protective wrapper.

LIMIT OF THE METHOD

The extreme sensitivity of gene amplification may cause false positive due to cross-contamination between samples and controls. Therefore, you should:

- physically separate all the products and reagents used for amplification reactions from those used for other reactions, as well as from post-amplification products,
- use tips with filters to prevent cross-contamination between samples,
- use disposable gloves and often change them,
- carefully open test tubes to prevent aerosol formation,
- close every test tube before opening another one.

The proper functioning of the amplification mix depends on the correct collection, correct transportation, correct storage and correct preparation of a biological sample.

As with any diagnostic device, the results obtained with this product must be interpreted in consideration of all the clinical data and other laboratory tests done on the patient.

As with any diagnostic device, there is a residual risk of obtaining invalid results, false positives and false negatives with this product.

STORAGE AND STABILITY

Store the master mix at -20°C, while the rest of the kit can be stored at +2+8°C.

An intact and well stored product has a stability of 9 months from the date of production.

Do not use beyond the expiration date which appears on the package label.

ANALITICAL PROCEDURE

FAST PCR:

1. prepare a number of PCR test tubes corresponding to the number of samples to be tested, plus a positive control and a negative control.
2. distribute to each test tube 17 µl of FAST PCR master mix.
3. add 3 µl of DNA extracted.
4. add to the positive control test tube 3 µl of the solution "positive control".
5. add to the negative control test tube 3 µl of the solution "negative control".
6. put the test tubes in the thermal cyclers and run the following programme:

cycles	denaturation	annealing/extension
1	95° C 1 min	
35	95° C 1 sec	70° C 10 sec
1		72° C 10 sec

The method is validated on thermal cyclers Applied Biosystem mod. 2700 and Veriti, Eppendorf, Techne mod. Progene, Biorad Genecycler.

Electrophoresis buffer preparation:

Dilute the TAE buffer in 2 litres of distilled water.

Detection:

1. remove the test tubes from the thermal cyclers.
2. move 10 µl of each amplification control specimen to an empty test tube.
3. move 10 µl of 100 bp ladder to an empty test tube
4. add 3 µl of loading solution to each test tube and carefully mix.
5. remove the pre-cast gel from its protection bag (cut the bag along three sides to prevent damages to the gel matrix). Do not touch the gel without gloves because it contains EtBr
6. immerse the pre-cast gel in the electrophoresis unit, after filling it with electrophoresis buffer.
7. slowly distribute the solution prepared during step 4 to the bottom of the gel wells.
8. run electrophoresis at 120 Volts for 15-30 minutes according to your apparatus.
9. put the gel on a transilluminator. Remove the plastic support on which it is stratified using a nylon-thread and take a picture of it. It is possible to take a picture without removing the plastic support, you must lean the gel on the transilluminator with the plastic support face up because it is not UV transparent.

INTERPRETATION OF RESULTS

The use of positive and negative control in each amplification session allows to verify the correct functioning of the amplification mix and the absence of any contamination.

Make sure that the negative control does not show a band identical to that appearing in the positive control to exclude any possibility of contamination or a false positive. The session is invalid and must be repeated from the amplification phase.

Ensure the correct amplification of the positive control represented by the 303 bp specific bands presence.

If the specific amplification product is absent in the positive control reaction, this means that problems have occurred during the amplification phase (inefficient or absent amplification) which may cause false negatives. The session is invalid and must be repeated from the amplification phase.

The appearance in the test samples of the 303 bp bands, verifiable by direct comparison with the positive control indicates the presence of Helicobacter pylori.

Low molecular weight bands, which can appear in all samples, are produced by unincorporated primers. Such are not significant for the interpretation of results.

PERFORMANCES

Analytical sensitivity:

For the purposes of this evaluation is considered as analytical sensitivity the highest serum dilution (title) to which a positive sample can be subjected without the system losing the ability to detect it as positive. The analytical sensitivity of the system was assessed by analyzing plasmid DNA (pTZ-HP), quantified by spectrophotometric analysis, containing the genomic region of interest in serial dilutions from 3,000 copies to 30 copies/reaction.

Concentration	30 copies	300 copies	3,000 copies
N° Test	9	9	9
N° Positives	0	9	9
N° Negatives	9	0	0

The Analytical sensitivity ≤ 300 copies reaction

Clinical sensitivity:

For the purposes of this evaluation is considered as clinical sensitivity the ability to determine true positives on the totality of positive samples screened. The analysis was performed on samples positive for Helicobacter pylori and the test was performed following the instructions in the package insert. Positive samples were confirmed with another system on the market. Results obtained showed a clinical sensitivity of 99.5%.

Analytical specificity:

The specificity of the test is guaranteed by the use of specific primers for the determination of Helicobacter pylori.

The alignment analysis of the regions chosen for the hybridization of specific primers for Helicobacter pylori with sequences available in database of the species specific antigen region shows their conservation, the absence of significant mutations and the complete specificity for the target analyzed.

Diagnostic specificity:

For the purposes of this evaluation is considered as diagnostic specificity the ability of the method to determine true negative samples. The specificity of the diagnostic system was evaluated by analyzing human genomic samples tested and confirmed negative with another system on the market.

Samples	
N° of Tests	38
Positives n°	0
Negative n°	38

Reproducibility and Repeatability:

The reproducibility and repeatability of the system was assessed by analyzing 2 dilutions (high and low) of plasmid DNA containing the genomic region of interest and quantified by spectrophotometric analysis (pTZ-HP) and 1 negative control (genomic DNA negative). 5 replicates are performed for each session for 3 different sessions performed by different operators.

		1	2	3	4	5
300,000 cps	lot 0	+++	+++	+++	+++	+++
	lot 1	+++	+++	+++	+++	+++
	lot 2	+++	+++	+++	+++	+++
300 cps	lot 0	+++	+++	+++	+++	+++
	lot 1	+++	+++	+++	+++	+++
	lot 2	+++	+++	+++	+++	+++
Negative	lot 0	---	---	---	---	---
	lot 1	---	---	---	---	---
	lot 2	---	---	---	---	---

Cross-Reactivity:

The alignment analysis of the regions chosen for the hybridization of specific primers for *Helicobacter pylori* with sequences available in database of the *species specific antigen region* shows their conservation, the absence of significant mutations and the complete specificity for the target analyzed.

It was also carried out analysis on positive samples for other gram-negative bacteria and the test was performed following the instructions for use.

Sample	Positive samples for	H. pylori (CL20)	H. pylori (CL20)	H. pylori (CL20)
1	<i>E.coli</i>	Negative	Negative	Negative
2	<i>Salmonella</i>	Negative	Negative	Negative
3	<i>Citrobacter</i>	Negative	Negative	Negative

INTERFERENCE

Verify that the extracted DNA from the sample base there are no mucoproteins and hemoglobin in order to exclude possible inhibition in the PCR reaction. Interference due to contaminants can be detected by spectrophotometric analysis and ratio of data obtained at 260 nm (absorption maximum of nucleic acids) and 280 nm (absorption maximum of proteins). A pure DNA should have a ratio of about 1.8.

QUALITY CONTROL

You should verify the correct extraction of DNA from test samples using the product CL54K - β -globin that allows the human β -globin gene amplification.

It's also suggested to include a negative and a positive sample tested already before or reference material, as an internal quality control of each session of extraction, amplification and detection.

REFERENCES

Varsha Singh, Shrutikirti Mishra, GRK Rao, Ashok Kumar Jain, V.K. Dixit, Anil Kumar, Gulati, Divya Mahajan, Michael McClelland, and Gopal Nath. Evaluation of Nested PCR in detection of *Helicobacter pylori* targeting a highly conserved gene: HSP60. References: *Helicobacter*. 2008 February ; 13(1): 30-34

Shrutikirti Mishra, Varsha Singh, GRKoteswar Rao, Ashok Kumar Jain, Vinod Kumar Dixit, Anil Kumar Gulati, and Gopal Nath. Detection of *Helicobacter pylori* in stool specimens: comparative evaluation of nested PCR and antigen detection. References: *Infect Developing Countries* 2008; 2(3): 206-210

TROUBLESHOOTING

Amplification signal in the negative control reaction









Possible causes	Solutions
Error displacing the DNA extract.	<ul style="list-style-type: none"> - Use only micropipettes and Sterile tips with aerosol filter - Wear protective clothing such as laboratory coats and disposable gloves while assaying samples - Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use - Use the positive control as the last sample - Use the negative control as the first sample
Contamination of the amplification mixes	<ul style="list-style-type: none"> - Don't dispense the amplification mix with the some pipettes used in the extraction or detection area - Use only micropipettes and Sterile tips with aerosol filter - Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use - Close the amplification mix and return it at -20°C before open the others sample tubes
Contamination of the preparation area for amplification reaction	<ul style="list-style-type: none"> - Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use

Failure or incorrect amplification of positive control

Possible causes	Solutions
Possible error in the dispensation of the positive control	<ul style="list-style-type: none"> - Dispense carefully the positive control - Check the calibration of micropipettes used - Verify that the amount of positive control used corresponds to the method described in the Analytical Procedure (3μl)
Positive Control Degradation	<ul style="list-style-type: none"> - Test the mixture through a new batch of positive control
Wrong heat protocol	<ul style="list-style-type: none"> - Check the thermal cycler setting
The reaction mix is maintained at room temperature for too long	<ul style="list-style-type: none"> - Keep the amplification mix on ice during the use and store it at -20 °C immediately after finishing the preparation of test samples
Error during the dispensation of the amplification product in the the detection phase	<ul style="list-style-type: none"> - Be careful when loading on the gel of amplified

Aspecific bands in the sample amplification reaction

Possible causes	Solutions
Wrong heat protocol	<ul style="list-style-type: none"> - Check the thermal cycler setting
Interfering Presence	<ul style="list-style-type: none"> - Repeat the extraction step carefully
Excess of extracted DNA in the reaction	<ul style="list-style-type: none"> - Evaluate the DNA concentration

	In vitro diagnostic device
	Read the instruction's manual
	Range of temperature
	Use within (dd/mm/yyyy: year-month)
	Lot (xxxx)
	Code
	Manufacturer
	Contains sufficient for <n> tests

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