RIDA®GENE Helicobacter pylori

REF PG2305

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1. Intended use

For *in vitro* diagnostic use. RIDA®GENE Helicobacter pylori is a multiplex real-time PCR for the direct, qualitative detection of *Helicobacter pylori*[1] and its resistance to clarithromycin from human native tissue biopsy material.

The RIDA®GENE Helicobacter pylori multiplex real-time PCR is intended for use as an aid in diagnosis of gastric infections caused by *Helicobacter pylori*.

2. Summary and explanation of the test

Helicobacter pylori (*H. pylori*) is a gram-negative rod-shaped bacterium which colonises the human gut. *H. pylori* increases the secretion of stomach acid and hence leads to different gastric infections such as Type B Gastritis, gastric ulcers or duodenal ulcers. Worldwide, *H. pylori* has a prevalence rate of 50 %, whereas the infection rate is higher in developing countries compared to developed countries. In Germany, about 33 million people are infected with *H. pylori* of which 10 – 20 % develop ulcers. While the *H. pylori* strain type 2 lacks the pathogenicity factors cag and VacA, an infection with *H. pylori* strain type 1 leads to gastroduodenal ulcers and in case of a chronic infection, significantly increases the risk of gastric cancer. To protect itself from gastric acid, *H. pylori* settles inside the gastric mucosa. Here, *H. pylori* splits urea by the enzyme urease to increase the pH value in its close surroundings.
Today, *H. pylori* is detected by microscopy or by using the helicobacter-urease assay from gastric biopsies. Other detection methods are antigen testing or breath tests. After diagnosis of *H. pylori*, different treatment measures are possible. Often, the "Triple Therapy" is used which consists of a combination of Amoxicillin, Clarythromycin and a proton pump inhibitor or Metronidazol, Clarythromycin and a proton pump inhibitor.[2] However, increasing clarithromycin resistance lowers the success rate of such a treatment by 30 %. Also, other more and more often occurring resistances against antibiotics such as Metronidazol or Levofloxacin (Fluoroquinolon) lead to higher failure in *H. pylori* eradication therapies.[3]

3. Test principle

The RIDA®GENE Helicobacter pylori is a multiplex real-time PCR for the direct, qualitative detection of *Helicobacter pylori* from human biopsy samples.
After DNA-isolation, amplification of gene fragments (if present) specific for *Helicobacter pylori* (16S rRNA) and a potential resistance to clarithromycin (23S rRNA) occurs.
The amplified targets are detected with hydrolysis probes, which are labeled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the presence of a target the probes hybridize to the amplicons. During the extension step the Taq-Polymerase breaks the reporter-quencher

proximity. The reporter emits a fluorescent signal which is detected by the optical unit of a real-time PCR instrument. The fluorescence signal increases with the amount of formed amplicons. The RIDA®GENE Helicobacter pylori assay contains an Internal Control DNA (ICD) as an internal control of sample preparation procedure and/or to determine possible PCR inhibition.

4. Reagents provided

Tab. 1: Reagents provided (Reagents provided in the kit are sufficient for 100 determinations)

Kit Code	Reagent	Amount		Lid Color
1	Reaction Mix	2x	1050 µl	yellow
2	Taq-Polymerase	1x	80 µl	red
D	Internal Control DNA	2x	1700 µl	orange
N	No Template Control	1x	450 µl	white
P	Positive Control	1x	200 µl	blue

5. Storage instructions

- Protect all reagents from light and store at -20 °C. All reagents can be used until the expiration date. After expiry the quality guarantee is no longer valid.
- Carefully thaw and fully defrost reagents before using (e.g. in a refrigerator at 2 – 8 °C).
- Reagents can sustain up to 20 freeze/thaw cycles without influencing the assay performance (e.g. after the first thawing separate it in aliquots and freeze immediately).
- During PCR preparation all reagents should be stored cold in an appropriate way (2 – 8 °C).

6. Additional necessary reagents and necessary equipment

The RIDA®GENE Helicobacter pylori multiplex real-time PCR assay is suitable for use with following extraction platforms and real-time PCR instruments:

Tab. 2 Necessary equipment

Extraction platforms	
R-Biopharm	RIDA® Xtract
Promega	Maxwell® RSC
Roche	MagNA Pure 96
Real-time PCR instruments	
Roche	LightCycler® 480II
Agilent Technologies	Mx3005P
Applied Biosystems	ABI 7500
Bio-Rad	CFX96™
QIAGEN	Rotor-Gene Q

Note: Only use 0.1 ml tubes on the Rotor-Gene Q (QIAGEN).

If you want to use other extraction platforms or real-time PCR instruments please contact R-Biopharm at mdx@r-biopharm.de.

- RIDA®GENE Color Compensation Kit IV (PG0004) for use with the LightCycler® 480II
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with a rotor for the reaction vials
- Vortexer
- Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1000 µl)
- Filter tips
- Powder-free disposal gloves
- PCR water (BioScience grade, nuclease-free)

7. Precautions for users

For *in vitro* diagnostic use.
This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories have to be followed. The instruction manual for the test procedure has to be followed. Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes. During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure. Do not smoke, eat or drink in areas where samples or reagents are being used.

- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- Samples must be treated as potentially infectious as well as all reagents and materials being exposed to the samples and have to be handled according to the national safety regulations.
- Do not use the kit after the expiration date.

All reagents and materials used have to be disposed properly after use. Please refer to the relevant national regulations for disposal.

For more details see Safety Data Sheets (SDS) at www.r-biopharm.com.

8. Collection and storage of samples

8.1 Sample preparation from biopsy material

For DNA isolation of biopsy samples, use a commercially available DNA isolation kit (e.g. RIDA® Xtract (R-Biopharm)) or DNA extraction system (e.g. Maxwell® RSC (Promega)). Extract DNA according to the manufacturer's instructions.
We recommend to incubate the biopsy material over night at 55 °C using Proteinase K before extraction. From this sample, use the appropriate volume for the extraction according to the manufacturer's instructions.

The RIDA®GENE Helicobacter pylori assay contains an Internal Control DNA that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient. The Internal Control DNA can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as PCR inhibition control.

If the Internal Control DNA is used only as a PCR inhibition control, 1 µl of the Internal Control DNA should be added to the Master-Mix (see Tab.4).

If the Internal Control DNA is used as an extraction control for the sample preparation procedure **and** as PCR inhibition control, 20 µl of the Internal Control DNA has to be added during extraction procedure. The Internal Control DNA should always be added to the specimen-lysis buffer mixture and must **not** be added directly to the specimen. We also recommend to add 1 µl of the Internal Control DNA to the negative control and positive control PCR Mix.

9. Test procedure

9.1 Master-Mix preparation

Calculate the total number of PCR reactions (sample and control reactions) needed. One positive control and one negative control must be included in each assay run.

We recommend calculating an additional volume of 10 % to compensate imprecise pipetting (see Tab. 3, Tab. 4). Thaw, mix gently and briefly centrifuge the Reaction Mix, the Taq-Polymerase, the Positive Control, the No Template Control and the Internal Control DNA before using. Keep reagents appropriately cold during working step (2 – 8 °C).

Tab. 3: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD as extraction and PCR inhibition control)

Kit code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.3 µl	212.3 µl
2	Taq-Polymerase	0.7 µl	7.7 µl
	Total	**20 µl**	**220 µl**

Mix the components of the Master-Mix gently and briefly spin down.

Tab. 4: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD only as PCR inhibition control)

Kit code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.3 µl	212.3 µl
2	Taq-Polymerase	0.7 µl	7.7 µl
D	Internal Control DNA	1.0 µl	11 µl
	Total	**21.0 µl**	**231.0 µl**

Mix the components of the Master-Mix gently and briefly spin down.

9.2 Preparation of the PCR-Mix

Pipette 20 µl of the Master-Mix in each reaction vial (tube or plate).

Negative control: Add 5 µl No Template Control to the pre-pipetted Master-Mix.

Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the PCR-Mix of the negative control.

Sample: Add 5 µl DNA extract to the pre-pipetted Master-Mix.

Positive control: Add 5 µl Positive Control to the pre-pipetted Master-Mix.

Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the PCR-Mix of the positive control.

Cover tubes or plate. Spin down and place in the real-time PCR instrument. The PCR reaction should be started according to the PCR instrument set-up see (Tab. 5, Tab. 6, Tab.7, Tab. 8).

9.3 PCR instrument set-up

9.3.1 DNA real-time PCR profile

Tab. 5: DNA real-time PCR profile for LightCycler® series and Rotor-Gene Q

Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	10 sec, 95 °C 15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

Tab. 6: DNA real-time PCR profile for Mx3005P, ABI7500, CFX96™

Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	15 sec, 95 °C 30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

9.3.2 Universal real-time PCR profile

Note: The universal real-time PCR profile should only be used for DNA assays when combining RIDA®GENE DNA and RNA real-time PCR assays in one run.

Tab. 7: Universal real-time PCR profile for LightCycler® series

Reverse Transcription	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	10 sec, 95 °C 15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

Tab. 8: Universal real-time PCR profile for Mx3005P, ABI7500, CFX96™ and Rotor-Gene Q

Reverse Transcription	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	15 sec, 95 °C 30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

9.4 Detection channel set-up

Tab. 9: Selection of appropriate detection channels

Real-time PCR instrument	Detection	Detection channel	Note
Roche LightCycler® 480II	*H. pylori*	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required**
	ICD	533/580	
	Clarithromycin resistance	618/660	
ABI 7500	*H. pylori*	FAM	**Check that passive reference option ROX is none**
	ICD	VIC	
	Clarithromycin resistance	Cy5	
Agilent Techn. Mx3005P	*H. pylori*	FAM	**Check that reference dye is none**
	ICD	HEX	
	Clarithromycin resistance	Cy5	
Qiagen Rotor-Gene Q	*H. pylori*	Green	**The gain settings have to be set to 5, according to the default settings**
	ICD	Yellow	
	Clarithromycin resistance	Red	
Bio-Rad CFX96™	*H. pylori*	FAM	**-**
	ICD	VIC	
	Clarithromycin resistance	Cy5	

10. Quality control

The analysis of the samples is done by the software of the used real-time PCR instrument according to the manufacturer's instructions. Positive control and negative control have to show correct results (see Table 10, Fig. 1, Fig. 2) in order to determine a valid run.

The Positive Control has a concentration of 10^3 copies/µl. In each PCR run it is used in a total amount of 5 x 10^3 copies.

Tab. 10: For a valid run, the following conditions must be met:

Sample	Assay result	ICD Ct	Target Ct
Positive control	Positive	NA *[1]	See Quality Assurance Certificate
Negative control	Negative	Ct > 20	0

*[1] *No Ct value is required for the ICD to make a positive call for the positive control.*

If the positive control is not positive within the specified Ct range but the negative control is valid, prepare all new reactions including the controls.

If the negative control is not negative but the positive control is valid, prepare all new reactions including the controls.

If the required criteria are not met, following items have to be checked before repeating the test:

- Expiry of the used reagents
- Functionality of the used instrumentation
- Correct performance of the test procedure

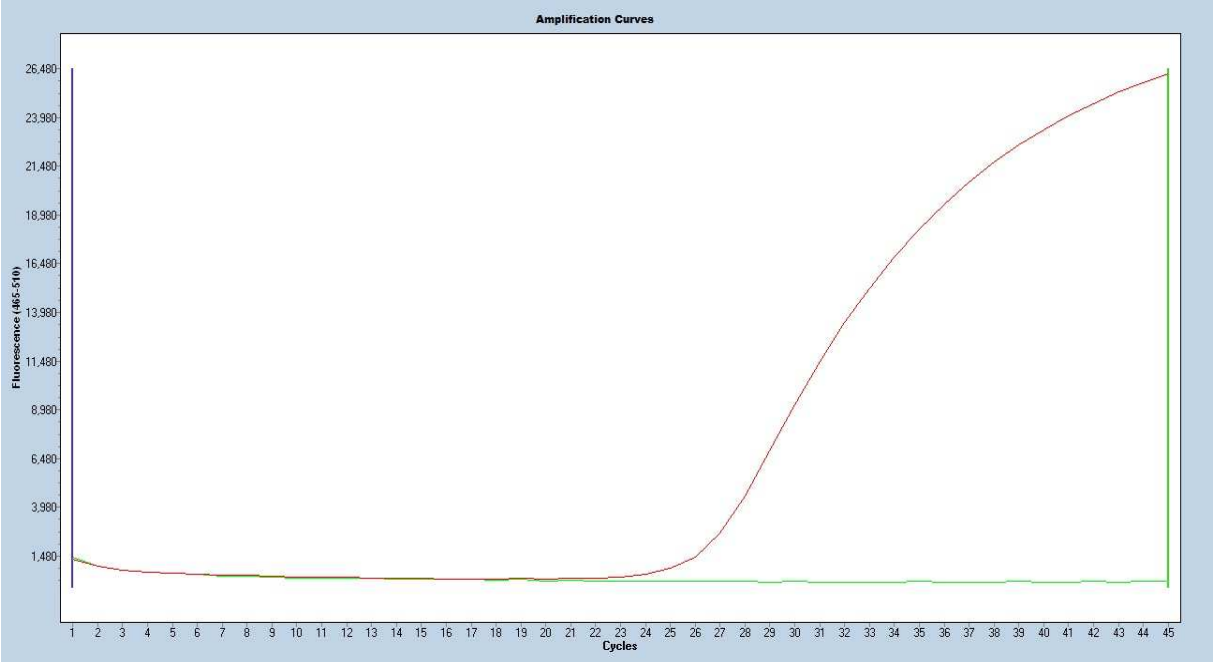

Fig. 1: Correct run of the positive and negative control (*Helicobacter pylori*) on the LightCycler® 480II

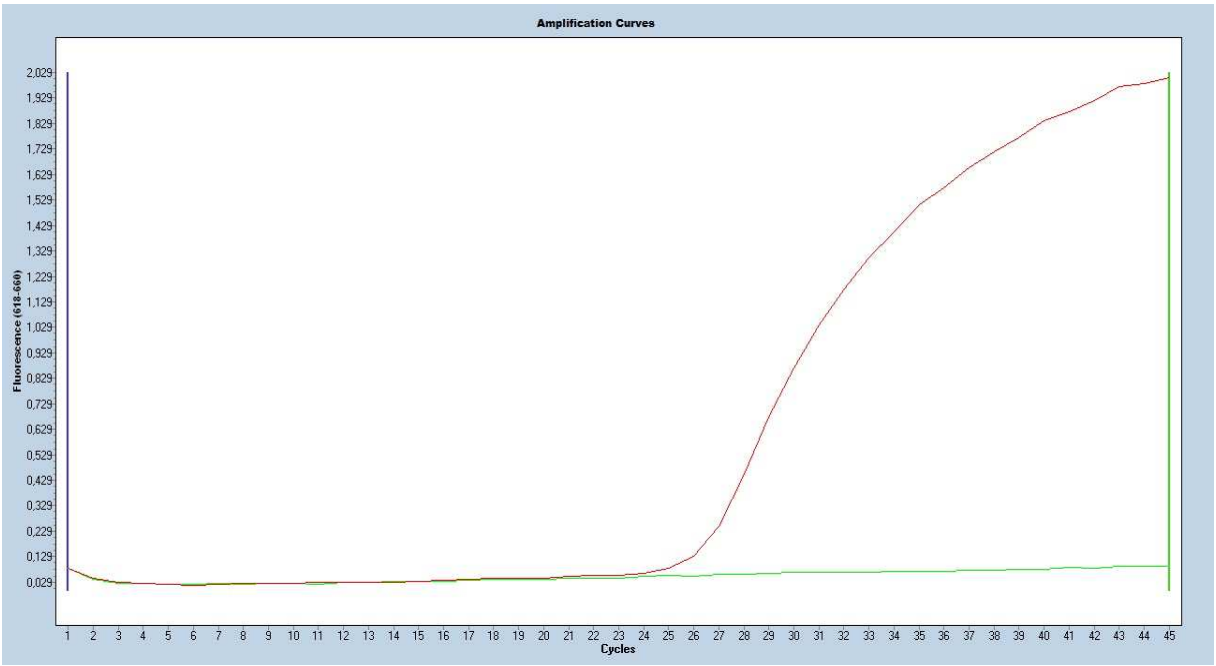

Fig. 2: Correct run of the positive and negative control (Clarithromycin resistance) on the LightCycler® 480II

11. Result interpretation

The result interpretation is done according to Table 11.

Tab. 11: Sample interpretation

Target genes			
Helicobacter pylori	**Clarithromycin resistance**	**ICD**	**Result**
positive	negative	**positive/negative**	***H. pylori* detected**
positive	**positive***	**positive/negative**	***H. pylori* and Clarithromycin resistance detected**
negative	**positive***	**positive/negative**	***H. pylori* not detected**
negative	negative	**positive**	**Target genes not detected**
negative	negative	negative	**Invalid**

***Note:** **When using the LightCycler® 480II (Roche) or the CFX96™ (Biorad), the fluorescence signal of a true positive signal in the clarithromycin resistance channel (Cy5) has to be more than 20 % of the fluorescence signal of the positive control or when using the Mx3005P (Agilent Technologies), the ABI 7500 (Applied Biosystems) or the Rotor-Gene Q (Qiagen), the fluorescence signal has to be more than 10 % of the fluorescence signal of the positive control. For a clearer evaluation, we recommend to set the threshold on this value (10 % or 20 % of the fluorescence signal of the positive control).**

A sample is evaluated negative, if the sample shows no amplification signal in the detection system, but the Internal Control DNA is positive. An inhibition of the PCR reaction or a failure in the extraction procedure can be excluded by the detection of the Internal Control DNA.

A sample is evaluated positive, if both, the sample and the Internal Control DNA, show an amplification signal in the detection system.

A sample is evaluated positive, if the sample shows an amplification signal in the detection system, but the Internal Control DNA is negative. The detection of the internal amplification control is not necessary, because high concentrations of the amplicon can cause a weak or absent signal of the internal amplification control.

A sample is evaluated invalid, if both, the sample and the Internal Control DNA show no amplification signal in the detection system. The sample contained a PCR inhibitor or a failure occurred in the extraction procedure. The extracted sample needs to be further diluted with PCR water (1:10) and re-amplified, or the isolation and purification of the sample has to be improved.

12. Limitations of the method

1. The result of molecular analysis should not lead to the diagnosis, but always be considered in the context of medical history and symptoms of the patient.
2. This assay is only validated for biopsy material.
3. Inappropriate specimen collection, transport, storage and processing or a pathogen load in the specimen below the analytical sensitivity can result in false negative results.
4. The presence of PCR inhibitors may cause invalid results.
5. Mutations or polymorphisms in primer or probe binding regions may affect detection of new variants resulting in a false negative result with the RIDA®GENE Helicobacter pylori assay.
6. As with all PCR based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection (LoD) may be detected, but results may not be reproducible.
7. A positive test result does not necessarily indicate the presence of viable organisms. However, a positive result is indicative for the presence of the target genes (16S rRNA, 23S rRNA).
8. In individual cases, weak cross-reactivity can occur in the Clarithromycin channel in presence of organisms that also carry the Clarithromycin wildtype genome but which are not *Helicobacter pylori*.
9. In individual cases, cross-reactivity can occur in the H. pylori channel in presence of *Helicobacter felis* though *H.pylori* is not present in the sample (cross-reactivity).

13. Performance characteristics

13.1. Clinical performance

In a retrospective clinical validation study we analyzed 225 and 139 human clinical specimens with the RIDA®GENE Helicobacter pylori assay compared to culture and a second commercial PCR method in an institute in Germany.

Tab. 12: Correlation of the *Helicobacter pylori* and Clarithromycin resistance results with the RIDA®GENE Helicobacter pylori multiplex real-time PCR and the reference method

Helicobacter pylori:

		Gold Standard			
		Positive	Negative	Total	
RIDA®GENE Helicobacter pylori	Positive	115	2	117	Sensitivity: 100 %
	Negative	0	108	108	Specificity: 98 %
	Total	115	110	225	

Clarithromycin resistance:

		Gold Standard			
		Positive	Negative	Total	
RIDA®GENE Helicobacter pylori	Positive	78	2	80	Sensitivity: 96 %
	Negative	3	56	59	Specificity: 97 %
	Total	81	58	139	

13.2 Analytical sensitivity

The RIDA®GENE Helicobacter pylori multiplex real-time PCR has a detection limit of ≥ 10 DNA copies per reaction.

The following figures 3 and 4 show a dilution series of *Helicobacter pylori* and the Clarithromycin resistance (each 10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II.

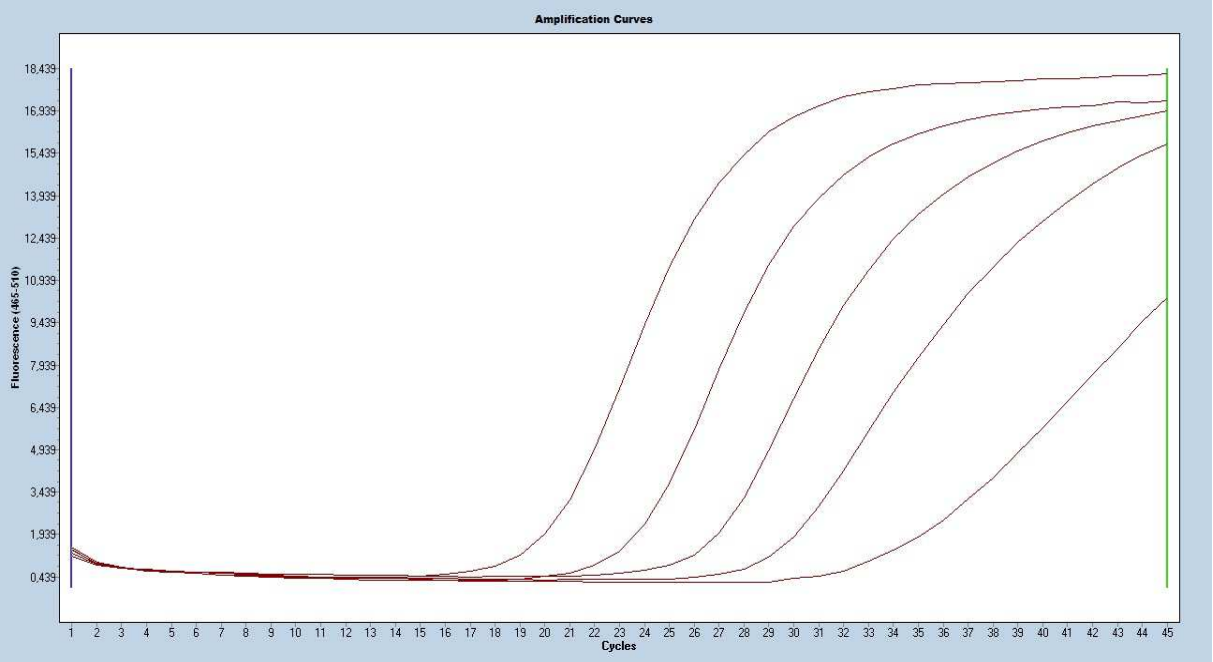

Fig. 3: Dilution series *H. pylori* (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

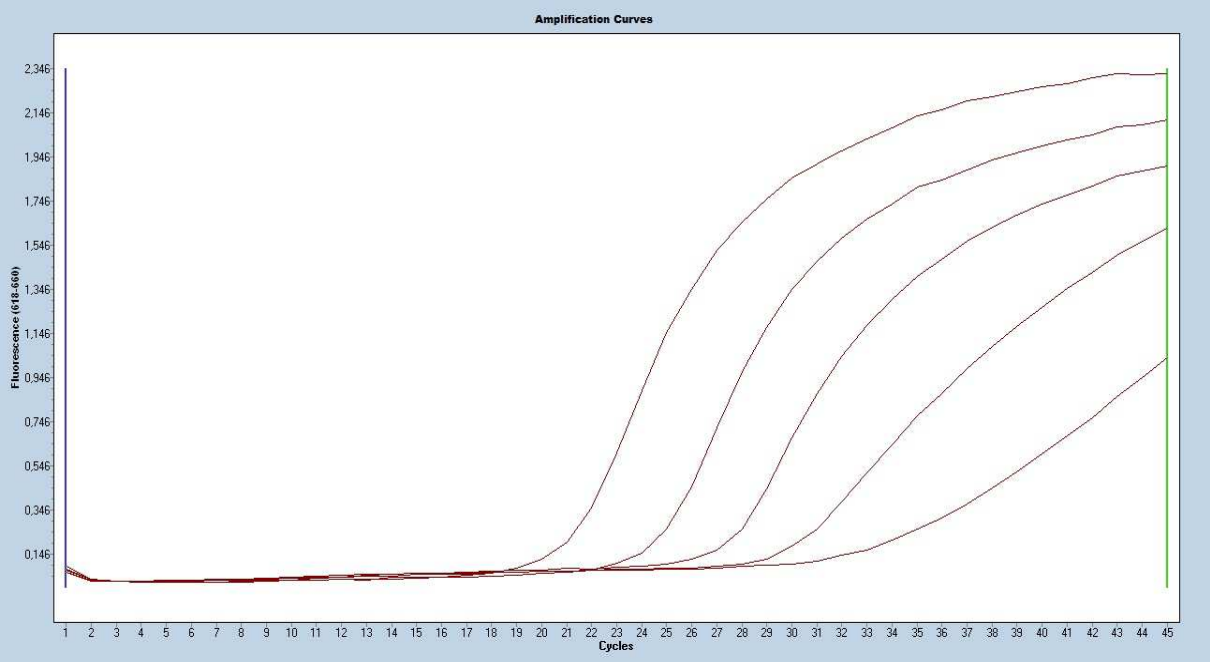

Fig. 4: Dilution series Clarithromycin resistance (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

The detection limit of the whole procedure depends on the sample matrix, DNA extraction and DNA concentration.

13.3 Analytical specificity

The analytical specificity of the RIDA®GENE Helicobacter pylori multiplex real-time PCR is specific for *Helicobacter pylori* from human biopsy samples. No cross-reaction could be detected for the following species (see Tab. 13):

Tab. 13: Cross-reactivity testing

Adenovirus 40, human, strain Dugan	-	*Campylobacter upsaliensis*	-	*Enterobacter cloacae*	-	*Pseudomonas aeruginosa*	-
Adenovirus 41, human, strain Tak	-	*Candida albicans*	-	*Enterococcus faecalis*	-	Rotavirus	-
Aeromonas hydrophila	-	*Citrobacter freundii*	-	*Giardia intestinalis* Portland 1	-	*Salmonella enteritidis*	-
Arcobacter butzleri	-	*Clostridium difficile*	-	*Giardia intestinalis* WB Clone C6	-	*Salmonella typhimurium*	-
Astrovirus	-	*Clostridium perfringens*	-	*Klebsiella oxytoca*	-	*Serratia liquefaciens*	-
Bacillus cereus	-	*Clostridium sordellii*	-	*Listeria monocytogenes*	-	*Shigella flexneri*	-
Bacteroides fragilis	-	*Cryptosporidium parvum*	-	Norovirus GGI	-	*Staphylococcus aureus*	-
Campylobacter coli	-	*E. coli* (O157:H7)	-	Norovirus GGII	-	*Vibrio parahaemolyticus*	-
Campylobacter jejuni	-	*E. coli* (O26:H-)	-	*Proteus vulgaris*	-	*Yersinia enterocolitica*	-
Campylobacter lari subsp. *lari*	-	*E. coli* (O6)	-				

14. Version history

Version number	Chapter and designation
2018-11-12	General revision 4. Reagents provided 5. Storage instructions 6. Additional necessary reagents and necessary equipment 8. Collection and storage of samples 9. Test procedure 10. Quality control 11. Result interpretation 12. Limitations of the method 13. Performance characteristics 14. Version history 15. Explanation of symbols

15. Explanation of symbols

General symbols

	For in vitro diagnostic use
	Consult instructions for use
	Lot number
	Expiry
	Store at
	Article number
	Number of tests
	Date of manufacture
	Manufacturer

Testspecific symbols

Not applicable

16. Literature

1. Rimbara E. PCR Detection of Helicobacter pylori in clinical samples. Methods Mol. Biol. 2013, 943: 279-287
2. Glocker E, *et al.* Quinolone resistance in Helicobacter pylori isolates in Germany. Antimicrob. Agents Chemother. 2007, 51(1): 346-349
3. O'Connor A. Treatment of Helicobacter pylori infection in 2010. Helicobacter ISSN 1523-5378, Helicobacter 15 (Suppl. 1): 46-52