RIDA®GENE Helicobacter pylori is a sensitive and specific real-time PCR test for the simultaneous detection and differentiation of Helicobacter pylori and its resistance to clarithromycin in human tissue biopsies

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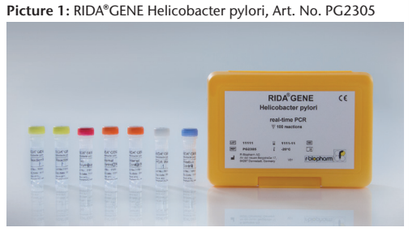
Objectives

Helicobacter pylori (H. *pylori*) increases the secretion of stomach acid and hence leads to different gastric diseases such as gastritis, duodenal ulcers and gastric cancer. The gold standard diagnostic procedure for *H. pylori* detection is culture including antimicrobial susceptibility testing. Other technologies include the helicobacter-urease assay from gastric biopsies, antigen testing from stool or breath tests.

After diagnosis of *H. pylori*, different treatment regimens are possible. The standard "triple therapy" consists of two antibiotics, mainly clarithromycin and amoxicillin, and a proton pump inhibitor (PPI). Increasing rates of resistant bacterial isolates lower the success rate by up to 30 %. In cases of treatment failure, culture with determination of the resistance pattern is mandatory. However, growth of *H. pylori* requires expertise and optimal pre-analytical conditions, but the rate of successful culture rarely exceeds 75 % in the routine microbiology lab. While culture and determination of the resistance pattern takes a minimum of 5 working days, results with real-time PCR can be obtained within 1 day.

This study aimed to evaluate the RIDA®GENE Helicobacter pylori real-time PCR assay for detection of *H. pylori* and its resistance to clarithromycin from native human biopsy samples.

Picture 1: RIDA®GENE Helicobacter pylori, Art. No. PG2305



Methods

The RIDA®GENE Helicobacter pylori real-time PCR assay simultaneously detects *H. pylori* specific DNA by targeting the 16S ribosomal rRNA gene and clarithromycin resistance by targeting the bacterial ribosomal DNA (23S rDNA). An internal control DNA detects PCR inhibition, monitors reagent integrity and confirms successful nucleic acid extraction.
In a retrospective study, 225 human gastric biopsies of which culture results were available were tested for *H. pylori* using the RIDA®GENE Helicobacter pylori real-time PCR. Results were compared to culture and a second commercial PCR method detecting *H. pylori* and its resistance to clarithromycin which is (Figure 1) herein referred to as reference method. In addition, 139 human tissue biopsies were investigated for presence of clarithromycin resistance. Proteinase K pre-treated tissue biopsies were extracted using the MagNAPure 96 (Roche) and extracted nucleic acids were analyzed on the Mx3005P (Agilent Technologies).

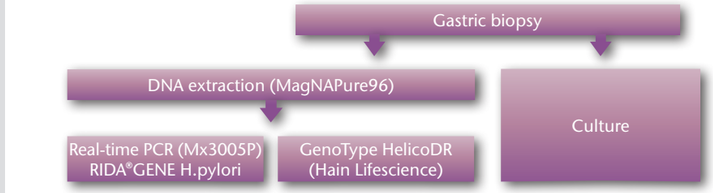


Fig. 1: Workflow study design RIDA®GENE Helicobacter pylori

Results

Overall, 225 clinical biopsy samples were tested. The RIDA®GENE Helicobacter pylori assay found 95 samples positive for *H. pylori* and 108 samples negative. Compared to culture, a total of 22 samples negative for *H. pylori* were positive with the RIDA®GENE Helicobacter pylori assay (Figure 2). Comparison with a second PCR method used as a reference method confrimed 20 positive *H. pylori* samples with only 2 discrepant samples remaining (Figure 4). Of the 225 characterised culture samples, only 129 samples could be tested against antibiotics indicating the limitations of this gold standard technology. Of the 129 samples, 63 samples were resistant to clarithromycin and 57 samples were clarithromycin-sensitive by the RIDA®GENE Helicobacter pylori assay and culture (Figure 3). Eight culture clarithromycin-sensitive samples were identified as being resistant by the RIDA®GENE Helicobacter pylori assay which was confirmed by the second PCR method (Figure 3).

Another two samples were clarithromycin-resistant by the second PCR method (Figure 5).
Compared to the reference method, consisting of culture and a second PCR method, an overall sensitivity of 100 % and specificity of 98 % of the RIDA®GENE Helicobacter pylori assay for detection of *H. pylori* was found (Figure 4). In addition, the RIDA®GENE Helicobacter pylori assay shows a sensitivity of 99 % and specificity of 96 % for detection of clarithromycin resistance of *H. pylori* in tissue biopsy samples (Figure 5).

Results obtained by culture and susceptibility testing took a minimum of 5 working days; results obtained using PCR were available within 1 working day.

H. pylori

		Culture +	Culture -	Total
RIDA®GENE H.pylori	+	95	22	117
	-	0	108	108
	Total	**95**	**130**	**225**

	CI (95 %)	
Sensitivity: 100 %	96 %	100 %
Specificity: 83 %	76 %	89 %

Fig. 2: Sensitivity and specificity of the RIDA®GENE Helicobacter pylori real-time PCR *versus* culture

Clarithromycin resistance

		Culture +	Culture -	Total
RIDA®GENE H.pylori	+	63	8	71
	-	1*	57	58
	Total	**64**	**65**	**129**

	CI (95 %)	
Sensitivity: 98 %	92 %	100 %
Specificity: 88 %	77 %	95 %

Fig. 3: Sensitivity and specificity of the RIDA®GENE Helicobacter pylori real-time PCR *versus* culture

* one sample was suspected to be a contamination in culture

H. pylori

		Reference method +	Reference method -	Total
RIDA®GENE H.pylori	+	115	2	117
	-	0	108	108
	Total	**115**	**110**	**225**

	CI (95 %)	
Sensitivity: 100 %	97 %	100 %
Specificity: 98 %	94 %	100 %

Fig. 4: Sensitivity and specificity of the RIDA®GENE Helicobacter pylori real-time PCR *versus* the reference method

Clarithromycin resistance

		Reference method +	Reference method -	Total
RIDA®GENE H.pylori	+	78	2	80
	-	3**	142	145
	Total	**81**	**144**	**225**

	CI (95 %)	
Sensitivity: 96 %	90 %	99 %
Specificity: 98 %	95 %	100 %

Fig. 5: Sensitivity and specificity of the RIDA®GENE Helicobacter pylori real-time PCR *versus* the reference method

** 2 samples were only positive by the second PCR method

Conclusion

The RIDA®GENE Helicobacter pylori assay can be reliably used to simultaneously identify a *H. pylori* infection and its clarithromycin resistance in tissue biopsies. Most importantly, culture-negative biopsies due to limitations were positively identified by the RIDA®GENE Helicobacter pylori assay and confirmed by a second PCR method. The use of the real-time PCR technology such as the RIDA®GENE Helicobacter pylori assay enables reliable diagnostics, especially in culture-negative samples, to identify *H. pylori* and its clarithromycin-resistance. Results are usually available within 1 working day, while culture and susceptibility testing require a minimum of 5 working days. Hence, real-time PCR detection enables fast and culture-independent decisions on guided therapy to ensure proper patient management.

03/2016