

Evaluation of two new molecular kits for detection of *Helicobacter pylori* and the mutations associated with clarithromycin resistance

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Introduction

Knowledge of antimicrobial susceptibility, especially to macrolides, has become crucial for the management of *Helicobacter pylori* infection. Our aim was to evaluate 2 new PCR kits able to detect *H. pylori* in gastric biopsies as well as the mutations associated with macrolide resistance.

Material & Methods

Samples: 200 successive biopsies received from gastroenterologists all over France.

Culture and antimicrobial susceptibility testing: culture was performed on Pylori agar (bioMérieux, Marcy-l'Etoile, France) and on in-house medium after grinding. The plates were incubated for 12 days before discarding them if there was no growth. Suspected colonies were tested for oxidase, catalase and urease. In the case of a positive culture, antimicrobial susceptibility testing was performed (MH 10% blood, 3 McF) including clarithromycin MIC determination by Etest.

Molecular biology tests performed:

- a validated in-house real-time PCR (FRET technology) (Oleastro M *et al.*, J Clin Microbiol 2003)
- two new kits: RIDA®GENE *Helicobacter pylori* (r-biopharm) and Amplidiag® *H. pylori*+ClariR (Mobidiag)
- discrepancies were solved by looking at *H. pylori* status determined by histopathology

Composite reference used:

- in case of a positive culture: patient categorized as *H. pylori* positive
- in case of a negative culture: patient categorized as *H. pylori* positive if the in-house PCR was positive or if the histopathology was positive for discrepancies

"RIDA®GENE *Helicobacter pylori*"



qPCR Instruments which can be used
 Roche: LightCycler® 480 (used in this study)
 Agilent Technologies: Mx3005P
 Applied Biosystems: ABI 7500
 Abbott: m2000rt Bio-Rad: CFX96™
 Cepheid: SmartCycler® QIAGEN: Rotor-Gene Q

"Amplidiag® *H. pylori*+ClariR"



qPCR Instruments which can be used
 Bio-Rad: CFX96™ (used in this study)
 Qiagen: RotorGene 6000
 Stratagene: MX3000

Results

1. Detection of *H. pylori*

1.a Results

Culture	In-house PCR	Ridagene PCR	Amplidiag PCR	N
+	+	+	+	68
-	+	+	+	5
-	-	+	+	1
-	-	-	+	2
-	-	-	-	124

Total N=200

1.b Status of false positives

The positive with Ridagene and Amplidiag only was positive by histological analysis → true positive

One of the 2 positives with Amplidiag only was positive by histological analyses → 1 true positive & 1 false positive

1.c Performance

	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
Ridagene	100	100	100	100
Amplidiag	100	99.2	98.6	100

2. Determination of macrolide resistance

2.a Results

AST (macrolide)	In-house PCR	Ridagene PCR	Amplidiag PCR	N
R	A2142-43G	A2142-43G	A2142-43G	22
S	Wild type	Wild type	Wild type	51

Total N=73

2.b Performance

✓ Perfect concordance

Conclusion

These two new kits have an excellent sensitivity and specificity, are convenient to use, adaptable to different thermocyclers, provide quick results and deserve to be used in *H. pylori* diagnosis.