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: RID®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

1. **Detection of *H. pylori***

1.a **Results**

<table>
<thead>
<tr>
<th>Culture</th>
<th>In-house PCR</th>
<th>Ridagene PCR</th>
<th>Amplidiag PCR</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>68</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>124</td>
</tr>
</tbody>
</table>

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

2. **Determination of macrolide resistance**

2.a **Results**

<table>
<thead>
<tr>
<th>AST (macrolide)</th>
<th>In-house PCR</th>
<th>Ridagene PCR</th>
<th>Amplidiag PCR</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>A2142-43G</td>
<td>A2142-43G</td>
<td>A2142-43G</td>
<td>22</td>
</tr>
<tr>
<td>S</td>
<td>Wild type</td>
<td>Wild type</td>
<td>Wild type</td>
<td>51</td>
</tr>
</tbody>
</table>

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.