**Supplementary Material**

**Supplementary Table 1.** Primers and probes used to amplify the target genes

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene** | **Forward primer** | | **Reverse primer** | **Length (bp)** |
| 16S rRNA | 5’-ctc att gcg aag gcg acc t-3’ | | 5’-tct aat cct gtt tgc tcc cca-3’ | 76 |
| ureA | 5’-cgt ggc aag cat gat cca t-3’ | | 5’-ggg tat gca cgg tta cga gtt t-3’ | 77 |
| vacA (s) | 5’-cgc aaa ats aat cgc cct-3’ | | 5’-gct gga atg atc acg gtb gt-3’ | 134 |
| Human -actin | 5’-aag tca gtg tac agg taa gcc-3’ | | 5’-gtc ccc caa ctt gag atg tat g -3’ | 83 |
| **TaqMan probe** | | **Sequence** | | |
| 16S rRNA | | 5’- (6-FAM)-att act gacgct gat tgcgcgaaagc-TAMRA- 3’ | | |
| ureA | | 5’- (6-FAM)-tcaggaaacatcgcttca ata ccc act t-TAMRA-3’ | | |

**Supplementary Table 2.** Number of positive results of each ddPCR triplicate test for vacA (s), ureA and 16S genes in *H. pylori*-positive patients by ddPCR according to the number of conventional positive tests.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gen** | **Number of**  **(+) replicates** | **Gene**  **result** | **0 DT *Hp* (+)**  **(n=57)** | **1 DT *Hp* (+)**  **(n=22)** | **2 DT *Hp* (+)**  **(n=9)** | **3 DT *Hp* (+)**  **(n=25)** |
| **VacA(s)** | 0/3 | - | 7 | 0 | 0 | 0 |
| 1/3 | - | 7 | 1 | 0 | 0 |
| 2/3 | + | 22 | 3 | 0 | 1 |
| 3/3 | + | 21 | 18 | 9 | 24 |
| **UreA** | 0/3 | - | 1 | 0 | 0 | 0 |
| 1/3 | - | 4 | 1 | 0 | 0 |
| 2/3 | + | 22 | 8 | 1 | 0 |
| 3/3 | + | 30 | 13 | 8 | 25 |
| **16 S** | 0/3 | - | 4 | 0 | 0 | 0 |
| 1/3 | - | 1 | 1 | 0 | 0 |
| 2/3 | + | 9 | 3 | 0 | 0 |
| 3/3 | + | 43 | 18 | 9 | 25 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gen** | **Number of**  **(+) replicates** | **Gene**  **result** | **0 DT *Hp* (+)**  **(n=101)** | **1 DT *Hp* (+)**  **(n=19)** | **2 DT *Hp* (+)**  **(n=3)** | **3 DT *Hp* (+)**  **(n=0)** |
| **VacA(s)** | 0/3 | - | 43 | 7 | 1 | 0 |
| 1/3 | - | 47 | 3 | 2 | 0 |
| 2/3 | + | 5 | 6 | 0 | 0 |
| 3/3 | + | 6 | 3 | 0 | 0 |
| **UreA** | 0/3 | - | 44 | 6 | 2 | 0 |
| 1/3 | - | 34 | 11 | 1 | 0 |
| 2/3 | + | 17 | 2 | 0 | 0 |
| 3/3 | + | 6 | 0 | 0 | 0 |
| **16 S** | 0/3 | - | 70 | 14 | 2 | 0 |
| 1/3 | - | 24 | 4 | 1 | 0 |
| 2/3 | + | 4 | 0 | 0 | 0 |
| 3/3 | + | 3 | 1 | 0 | 0 |

**Supplementary Table 3.** Number of positive results of each ddPCR triplicate test for vacA (s), ureA and 16S genes in *H. pylori-*negative patients by ddPCR according to the number of conventional positive tests

# Supplementary Table 4: *H. pylori* copies of genome equivalents detected by ddPCR in dyspeptic patients. Results are expressed as mean ± SEM: \* p< 0.05/ \*\*p< 0.01/ \*\*\*p< 0.005 / \*\*\*\*p< 0.001 vs. 0 DT *Hp (+).*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | 0 DT *Hp*(+) | 1 DT *Hp*(+) | 2 DT *Hp*(+) | 3 DT *Hp*(+) |
| vacA s | 2.9 + 0.3 | 63.6 + 30.4 | 533 + 481\*\*\* | 1881 + 1091\*\*\*\* |
| UreA | 3.6 + 0.2 | 86.4 + 34.5\* | 315 + 210\*\*\* | 1110 + 312\*\*\*\* |
| 16S | 8.2 + 1 | 221.7 + 94\*\* | 951 + 759\*\*\* | 6410 + 3949\*\*\*\* |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **0 DT *Hp*(+) (n=57)** | **1 DT *Hp*(+)(n=22)** | **2 DT *Hp*(+)(n=9)** | **3 DT *Hp*(+)(n=25)** |
| **Gastritis (no/yes/no data)** | 1/54/2 | 2/20/0 | 0/9/0 | 0/25/0 |
| **Gastritis activity (no/yes/no data)** | 53/2/2 | 15/7/0 | 1/8/0 | 1/24/0 |
| **Gastritis severity (0/1/2/ no data)** | 1/54/0/2 | 2/17/3/0 | 0/7/2/0 | 0/15/10/0 |
| **Lymphoid follicle (no/yes/no data)** | 43/11/3 | 10/12/0 | 1/6/2 | 8/15/2 |
| ***Helicobacter pylori*** | 0 | 4 | 7 | 25 |
| **Metaplasia/ atrophy** | 0/0 | 0/1 | 0/0 | 0/0 |

**Supplementary Table 5**. Histological diagnosis of *H. pylori*-positive patients by ddPCR according to the number of positive conventional tests.

**Supplementary Table 6.** Histological diagnosis of *H. pylori-*negative patients by ddPCR according to the number of positive conventional tests

|  |  |  |  |
| --- | --- | --- | --- |
|  | 0 DT *Hp*(+) (n=101) | 1 DT *Hp*(+) (n=19) | 2 DT *Hp*(+) (n=3) |
| Gastritis (no/yes/no data) | 10/90/1 | 0/19/0 | 0/3/0 |
| Gastritis activity (no/yes/no data) | 97/2/2 | 17/2/0 | 2/1/0 |
| Gastritis severity (0/1/2/ no data) | 11/87/2/1 | 1/18/0/0 | 0/2/1/0 |
| Lymphoid follicle (no/yes/no data) | 77/18/6 | 14/5/0 | 3/0/0 |
| *Helicobacter pylori* | 0 | 0 | 1 |
| Metaplasia/ atrophy | 0/1 | 0/2 | 0/0 |

**Supplementary Table 7.** Results of the individual conventional diagnostic tests *according to H. pylori* status determined by ddPCR

|  |  |  |  |
| --- | --- | --- | --- |
| **ddPCR (+)** (n)  0 DT *Hp*(+) (57)  1DT *Hp*(+) (22)  2 DT *Hp*(+) (9) | **Histology (+)** | **RUT (+)** | **UBT (+)** |
| 0 | 0 | 0 |
| 4 | 1 | 17 |
| 7 | 3 | 8 |
| **ddPCR (-)** (n)  0 DT *Hp*(+) (101)  1 DT *Hp*(+) (19)  2 DT *Hp*(+) (3) | **Histology (+)** | **RUT (+)** | **UBT (+)** |
| 0 | 0 | 0 |
| 0 | 0 | 19 |
| 1 | 2 | 3 |

**ddPCR thermal cycling conditions**

The ddPCR was performed using the QX200™ Droplet Digital™ PCR System (Bio-Rad). Droplets, partitioning the EvaGreen or TaqMan reaction mix, were generated by a QX200 Droplet Generator (Bio-Rad).After transferring the generated drops to a 96-well PCR plate, end-point PCR was carried out on a Bio-Rad T100 thermocyclerwith the following thermal cycling conditions:

1. Eva Green for the vacA (s) gene. PCR was performed with an initial enzyme activation step at 95°C for 5 min, followed by 40 cycles of [denaturation (95°C for 30 s) and annealing (60°C for 1 min) (ramp rate set to 2°C)], and finally a stabilization signal (4°C, 5 min. followed by 90°C 5min).
2. TaqManPCR for the ureA and 16S rRNA genes PCR was performed with an enzyme activation step at 95°C for 10 min, followed by 40 cycles of [denaturation(95° C for 30 s) and annealing (59°C for 1 min) (ramp rate set to 2°C)] and an enzyme deactivation step (98°C for 10 min).