Polymerase chain reaction versus culture in the diagnosis of *Helicobacter pylori* infection

Rune Johannessen,[1] Kåre Bergh,[2,3] Constantin Jianu,[4] Per Martin Kleveland[1,4]

[1]Unit for Gastroenterology, Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim; [2]Department of Laboratory Medicine, Children's and Women's Health, Norwegian University of Science and Technology, Trondheim; [3]Department of Medical Microbiology, Clinic of Laboratory Medicine, St. Olavs Hospital, Trondheim University Hospital, Trondheim; [4]Department of Gastroenterology, Clinic of Medicine, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway

Abstract

In the management of *Helicobacter pylori*-induced gastroduodenal disease, a pilot study at our hospital (St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway) revealed that culture often seemed to fail compared to the polymerase chain reaction (PCR). A more thorough evaluation was therefore undertaken. We included 201 patients referred to upper gastrointestinal endoscopy in the period 2002-2004. Serology, biopsy rapid urease test, culture and PCR were performed. Conventional PCR was performed using the *ureC*, *vacA* and *cagA* genes, and real-time PCR for *ureC*. A diagnostic standard was defined on the basis of all four tests, and all four tests were then compared to this standard. One hundred eleven patients were deemed *H. pylori*-positive by the defined diagnostic standard, and 90 were labeled negative. Compared to this standard, culture showed a sensitivity of 87.4%, which was significantly lower than PCR at 99.1% (P<0.001). Culture showed a perfect specificity of 100%, which was significantly better than PCR at 97.8%. *ureC* was the gene with the best sensitivity (94.6% in conventional PCR, 97.3% in real-time PCR). *vacA* sensitivity was 87.4%, which is significantly lower than *ureC* (P<0.001). *cagA* was present in 37.8% of our *H. pylori*-positive patients. By real-time PCR a significantly lower cycle threshold was observed in antral biopsies than in corpal biopsies, indicating a higher *H. pylori* DNA template concentration in antral biopsies. PCR-testing for *H. pylori* is faster and significantly more sensitive than culture. Culture on the other hand was significantly more specific than PCR in our hand.

Introduction

Diagnostic methods for the detection of *Helicobacter pylori* (*H. pylori*) are traditionally divided into invasive and non-invasive techniques based upon the need for endoscopy. The choice of test depends mostly on clinical situation, availability, population prevalence of infection and factors such as the use of antibiotics and proton pump inhibitors which may influence test results. Advantages of non-invasive tests are mainly their non-invasiveness and their independency of bacterial colonization density and distribution variability of the pathogen in the gastric mucosa.[1] *H. pylori* can be detected non-invasively by serology,[2] stool antigen tests and by urea breath testing.[3,4]

Gastroscopy, nevertheless, is often indicated in the management of *H. pylori* induced gastroduodenal disease. Biopsy histology provides essential information on the status of the mucosa and biopsy-based culture detects *H. pylori* with high specificity and under optimal conditions with excellent sensitivity.[5] Besides diagnosing active infection, culture also offers the possibility of performing resistance testing for a variety of antimicrobial agents.[1] Nevertheless, the sensitivity of biopsy-based tests and culture is significantly influenced by the density, viability and distribution of bacteria in the gastric mucosa.[6] In certain circumstances, *e.g.* short time after use of antibiotics, recent or ongoing use of proton pump inhibitors (PPI), and in patients with gastric mucosal atrophy, biopsy based methods may fail due to biopsy sampling error.[1] Culture can also fail due to incorrect transportation, processing and culturing conditions.[1] Genetic methods can also be used to detect *H. pylori* in gastric biopsies and are recently shown to increase sensitivity of *H. pylori* detection compared to culture.[7,8]

Besides accurately detecting *H. pylori* encompassing also non-culturable coccoid forms,[9] polymerase chain reaction (PCR) can be employed for strain characterization by detecting genes encoding pathogenic factors and identifying certain resistance traits by detecting microbial gene mutations.[10] PCR entails no special requirements in processing and transport, enabling a rapid and probably a cost-effective diagnosing of *H. pylori*, overcoming the delay associated with conventional culture methods.[10-12]

About ten years ago, after having used a conventional PCR method as a supplement to our standard *H. pylori* culture in gastric mucosal biopsy specimens, a preliminary investigation in our hospital disclosed that culture often seemed to fail compared to PCR. Culture gave a negative result in as much as 27% of PCR-positive biopsies, whereas 5% of positive cultures were found to have a negative PCR-result, necessitating a more thorough evaluation of the biopsy-based diagnostic methods in use. We aimed primarily to compare culture and PCR. Biopsy rapid urease test (RUT) and serology were added to the protocol in order to evaluate conflicting results between culture and PCR. Secondarily, we wanted to investigate how the three gene sequences selected (*ureC, vacA, cagA*) were suited for detecting and characterizing *H. pylori* colonization. Finally, we aimed to assess whether real-time PCR was more accurate than conventional PCR.

Correspondence: Rune Johannessen, Clinic of Medicine, St. Olavs Hospital, N-7006 Trondheim, Norway. Tel. +47.93009665.
E-mail: rune.trondheim@gmail.com

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Materials and Methods

Patients

Two hundred one patients consecutively recruited from patients referred for upper gastrointestinal endoscopy (UGE) in the period 2002-2004 were included. Evidence of *H. pylori* infection was sought by PCR, culture and a rapid urease test (RUT) of gastric biopsies and serology.

Upper gastrointestinal endoscopy

UGE was done using GIF 140 gastroscopes (Olympus, Japan) and topical (spray) lidocaine anesthesia only, after an overnight fast. In total six biopsies were taken from the mucosa of the greater curvature; three from both antrum and corpus; one for rapid urease testing and two for culture and PCR. Single-use biopsy forceps were used, and the endoscopes were manually and mechanically cleaned using glutaraldehyde as disinfectant, followed by a machine wash using Olympus Europe equipment.

H. pylori serology

Whole blood samples were drawn and sera separated and stored at -20°C until analyzed. IgG antibodies to *H. pylori* were determined by ELISA (Enzyme-Linked ImmunoSorbent Assay) using the commercial Pyloriset® EIA-G (Orion Diagnostics, Espoo, Finland).[2]

Rapid urease test

Urease testing was performed using the Hut-Test® (AstraZeneca, Sweden).[13] The dual biopsy-specimen urease tests were observed in an incubator at 37°C and read at 1 hour; and if not positive read again at latest after an additional 24 hours according to the manufacturer's recommendations.[13]

Microbiological methods

Culture

Biopsy specimens were placed and transported in Stuart's *medium* to the microbiology department and processed the same day. Biopsies for culture were ground in 250-300 μL of saline using a mortar and pestle. For culture 100 μL was seeded onto a selective agar and incubated in a microaerophil environment as described previously.[5] The agar plates were incubated for a period of ten days.

Polymerase chain reaction

After grinding the biopsy as described above, the suspension was subjected to heat lysis in an Eppendorf tube on a heating block for 15 min at 95 °C. 2 μL of the lysate was used as template for PCR. Conventional PCR targeting the *ureC*, *vacA* and *cagA* genes was performed using PCR conditions as described elsewhere.[10,14,15] Real-time PCR for ureC was performed using the same primers as for conventional PCR and by constructing a TaqMan probe. Details of DNA primer and probe sequences are given in Table 1. PCR was run on a LightCycler® (Roche Molecular Diagnostics, Switzerland) platform using the Light-Cycler-Fast Start DNA Master Hybridazation Probe kit. Final concentrations of primers, probe and $MgCl_2$ were 0.5 μM, 0.2 μM, and 4 mM, respectively. PCR conditions were: 95°C for 5 min to activate the Taq polymerase, and 45 cycles of 5 sec at 95°C, 10 sec at 55°C and 20 sec at 72°C. To control for inhibition of PCR in biopsy specimens a real-time PCR targeting human DNA was employed as described elsewhere.[16]

Diagnostic standard definition

Even though many tests for diagnosing *H. pylori* exist, no single test can be considered a gold standard. In the current study, a diagnostic standard was defined on the basis of the combined results from culture, PCR, RUT and serology.[17]

A test was defined as *H. pylori* positive if at least one of the following conditions were present: i) all 4 tests positive; ii) 3 tests positive, 1 test negative or missing/not performed; iii) PCR and culture positive, RUT and/or serology negative or missing/not performed.

Conversely, a test was considered negative if at least one of the following conditions were present: i) all 4 tests negative; ii) 3 tests negative, 1 test positive or missing/not performed; iii) PCR and culture negative, RUT and/or serology positive or missing/not performed.

Four cases did not meet any of the above listed criteria for *H. pylori* status. These patients are described in detail under results, and labeled as negative or positive on the basis of all available information. Culture, PCR, RUT and serology were then compared to this defined diagnostic standard (Table 2).

Statistical analysis

Test accuracies are proportions, and were therefore compared using the χ^2-test. The level of significance was set to $P<0.05$. Mean Ct-values in antral and corpal biopsies were compared with a paired Student's t-test. All analyses were performed using SPSS (Statistical Package for the Social Sciences) versions 14.0-18.0.2.

Ethics

This study was approved by the regional ethics committee of the Norwegian University of Science and Technology in Trondheim, Norway. Informed consent was obtained from all participants.

Results

Our material comprised 201 patients with a mean age of 60 years (range 15-92), 93 of who were women (46%). Results from PCR and culture were registered for all 201 patients. Valid results from serology and rapid urease test were recorded in 188 (94%) and 183 (91%) patients, respectively (Table 3). In the remaining cases these two analyses were either not performed, or the results could not be retrieved. All patients had valid results for at least three tests.

In total, 111 patients were labeled as *H. pylori* positive by our defined diagnostic standard. Seventy-four of these were positive by all 4 tests, while 35 had 3 positive tests. Two patients did not meet the defined criteria for *H. pylori* positivity, but were labeled as positive after review of all available information. The first patient, a 76 years old man, had not gone through any prior anti-*H. pylori* (anti-HP) treatment, and was referred to endoscopy because of heartburn, acid regurgitation, epigastric pain and anemia with melena. He had a comorbidity of chronic obstructive pulmonary disease. Endoscopy showed esophagitis grade C (Los Angeles classification) and a fibrin-covered duodenal ulcer. RUT was negative, but serology positive with a titer of 88.

Table 1. Details of DNA sequences employed as primers and TaqMan probe for the detection of Helicobacter pylori in biopsy specimens.

Target gene	Primer and probe sequence (5'-3')	Product size (bp)	Reference
*ureC**	AAGCTTTTAGGGGTGTTAGGGGTTT AAGCTTACTTTCTAACACTAACGC 6-FAM-CGATTGGGGATAAGTTTGTGAGCG–TAMRA	294	14 This study
vacA	GAGCGAGCTATGGTTATGAC ACTCCAGCATTCATATAGA	229	10
cagA	AATACACCAACGCCTCCAAG TTGTTGCCGCTTTTGCTCTC	400	15

**ureC* is now termed gene for phosphoglucosamine mutase; bp, base pairs.

Culture was negative for antral and corpal biopsies. PCR was positive in both biopsies for the *ureC* gene, but negative in both biopsies for *vacA* and *cagA*. The second patient, also male, was aged 22 years and presented with a 2-year long history of epigastric pain and heartburn. He had not been using any medication. Endoscopy showed esophagitis grade B (Los Angeles classification) and antral gastritis. Culture was negative in both biopsies, while RUT was positive after 1 hour. Serology was not performed. PCR was positive for all three genes in antral and corpal biopsies.

In total, 90 patients were labeled as *H. pylori* negative by our defined diagnostic standard. Twenty-five of these were negative by all 4 tests, while 47 had 3 negative tests; 16 had negative PCR and culture, and positive or missing RUT and/or serology; 2 patients did not meet the defined criteria for *H. pylori* negativity, but were labeled as negative after a review of all available information. The first patient, a man aged 35 years, had gone through anti-HP treatment because of peptic ulcer three years earlier, and now presented with dyspepsia. He had been using H2RA recently, in addition to penicillin tablets towards an airway infection during the last week before the endoscopy, which showed a slight injection of the bulbus duodeni. Serology was positive with a low titer of 27, and RUT was negative. Culture was negative for antral and corpal biopsies. Antral biopsies were weakly PCR-positive for *ureC* and *vacA*, and negative for *cagA*. Corpal biopsies were PCR-negative for all three genes. The second patient, also male, was 60 years of age, without any known prior anti-HP therapy, presenting with heartburn. Endoscopy showed esophagitis grade B (Los Angeles classification) and antral gastritis. RUT was positive after 24 hours, while serology was negative. Culture was negative for antral and corpal biopsies. PCR was positive in antral biopsies for *ureC* and *vacA*, but negative for *cagA*. Corpal biopsies were negative for all three genes.

Compared to the defined diagnostic standard, PCR had a sensitivity of 99.1%, culture 87.4%, RUT 97.1% and serology 96.0% (Table 2). Culture was thus falsely negative in 12.6% of the patients, and PCR in 0.9%. Culture had perfect specificity of 100%, PCR 97.8%. PCR was significantly more sensitive than culture, RUT and serology (P<0.001). *ureC* was more sensitive than *vacA* and *cagA* (P<0.001). There was a tendency towards better sensitivity for real-time PCR compared with conventional PCR for *ureC*, but this difference did not reach statistical significance. No inhibition of PCR was evident in any of the biopsies investigated.

The prevalence of atrophic gastritis increases with age, and decreases bacterial density of *H. pylori* in the gastric mucosa.[18] Recent anti-HP or PPI treatment is also known to be associated with a lower bacterial density in the gastric mucosa.[1] An *ad-hoc* subanalysis was done to compare results in subgroups of patients with presumably different bacterial densities (Tables

Table 2. Test accuracy when compared to a defined diagnostic standard.

Variable	Sensitivity (%)	Specificity (%)
Serology	96.0	63.2
Rapid urease test	97.1	55.7
Culture	87.4	100
PCR total	99.1	97.8
Conventional PCR *cagA*	37.8	100
Conventional PCR *vacA*	87.4	97.8
Conventional PCR *ureC*	94.6	97.8
Real-time PCR *ureC*	97.3	97.8

PCR, polymerase chain reaction. PCR total means result for all PCR analyses together. Diagnostic standard: see definition under methods.

Table 3. Results of different diagnostic methods.

Variable	N pos	%	N neg	%	N total
Serology	129	69	59	31	188
Rapid urease test	136	74	47	26	183
Culture	97	48	104	52	201
PCR total	112	56	89	44	201
Conventional PCR *cagA*	42	21	159	79	201
Conventional PCR *vacA*	99	49	102	51	201
Conventional PCR *ureC*	107	53	94	47	201
Real-time PCR *ureC*	110	55	91	45	201

PCR, polymerase chain reaction. PCR total means result for all PCR analyses (positive test means that at least one gene was positive).

Table 4. Test accuracies in patients with presumably different bacterial densities, when compared to a defined diagnostic standard.

	Sensitivities			Specificities		
	High density (n=153)	Low density (n=48)		High density (n=153)	Low density (n=48)	
Test type	Sens (%)	Sens (%)	Sign	Spec (%)	Spec (%)	Sign
Serology	95.5	100	<0.001	70.9	50.0	0.002
Rapid urease test	97.8	92.9	ns	60.0	48.3	ns
Culture	89.7	71.4	<0.001	100.0	100.0	ns
PCR total	99.0	100.0	<0.001	98.2	97.1	ns
Conventional PCR *cagA*	39.2	28.6	0.021	100.0	100.0	ns
Conventional PCR *vacA*	88.7	78.6	0.015	98.2	97.1	ns
Conventional PCR *ureC*	95.9	85.7	0.004	98.2	97.1	ns
Real-time PCR *ureC*	96.9	100	<0.001	98.2	97.1	ns

Sens, sensitivity; Spec, specificity; Sign, significance level of difference (P-value), based on χ^2-test; ns, non-significant P-value (*i.e.* P>0.05); PCR, polymerase chain reaction.
Diagnostic standard: see definition under methods; High density: patients with a presumable high density of bacteria in the gastric mucosa, *i.e.* primary diagnostics (n=156); Low density: patients with a presumable low density of bacteria in the gastric mucosa, *i.e.* having received recent anti-HP or PPI therapy (n=45).

4 and 5). In patients that had received anti-HP or PPI treatment recently, culture showed a sensitivity of 71.4%. The sensitivity in the opposite subgroup was 89.7%, a difference which was highly significant (P<0.001) (Table 4). Comparing age groups, we found culture to be less sensitive in older patients (>75 years), with a significant P-value of 0.016 (Table 5).

One hundred two patients had a positive real-time PCR biopsy in both the antrum and the corpus. 95 of these had retrievable data for quantification, *i.e.* the cycle threshold (Ct) value. Lower Ct value indicates higher bacterial density in the biopsy. Mean Ct value for antrum biopsies was 30.65 (SD 3.60), and 31.52 in the corpus (SD 3.23). A paired t-test showed that this difference of 0.87 units is statistically significant with a P-value of 0.01 (Table 6). In subgroups of patients with presumably high bacterial densities, an even greater difference in the same direction was found, while there was no significant difference in subgroups with a presumably low bacterial density (Table 6).

Discussion

This study compared culture and PCR for detecting *H. pylori*, to a diagnostic standard which was based on the combined results of culture, PCR, serology and RUT. PCR was found to be significantly more sensitive than culture (P<0.001). Culture was found to be falsely negative in 12.6% of the cases, PCR only in 0.9%. Culture was on the other hand found to have a perfect specificity, where as PCR was deemed falsely positive in 2.2% of the cases.

The results are in accordance with earlier studies comparing conventional PCR and culture and recent studies using improved PCR-methods, including quantitative real-time PCR, showing PCR to be far more sensitive than culture in detecting current *H. pylori* infection.[1,8,19-22]

Although culture from biopsy specimens has the potential of leading to a high sensitivity (>90%) under optimal conditions,[1,5,12] the literature reports sensitivity values of culture varying from 50% to 70% in experienced laboratories.[1,22] Our sensitivity (87.4%) seems to be comparable or better than the average of published values,[1] but still renders 12.6% falsely negative results, only reflecting the fastidious character of the bacterium and the inevitable suboptimal accuracy of the method in detecting active *H. pylori* infection in this type of unselected patient material. Accordingly, in an ad hoc subanalysis, culture was found to be significantly less sensitive when used in older patients (>75 yrs) (Table 4), and in patients having had recent anti-HP therapy or PPI treatment (Table 5). This is in accordance with several other studies indicating that the infection in such patients has an uneven mucosal distribution of bacteria.[1,18]

Real-time PCR performed well regardless of age or recent anti-HP- or PPI-therapy (Tables 4 and 5). One patient was culture positive and PCR negative, while both serology and RUT were positive. This reflects a false negative PCR reaction, probably indicating that the number of bacteria was under the threshold of detection in the specimen investigated. The cultured strain did contain the *ureC* gene. In general, other reasons for a negative PCR would include polymorphism of the gene targeted or Taq polymerase inhibitors.[23] Two patients in this study were deemed as false positive by PCR. False positive PCR reactivity, however seldom, is theoretically possible, probably by mechanism of the assay targeting exogenous DNA sharing genes with the PCR primers.[11,23] That could occur in the presence of close relatives of *H. pylori* such as *H.heilmannii* in gastric mucosa, Campylobacter species originating from the oral cavity, gastric mucosal segments of *H. pylori* DNA from earlier treated infection, or contamination of material.

Quantitative results from real-time PCR in

Table 5. Test accuracies in different age groups, when compared to a defined diagnostic standard.

	Sensitivities			Specificities		
	≤75 yrs (n=158)	>75 yrs (n=43)		≤75 yrs (n=158)	>75 yrs (n=43)	
Test type	Sens (%)	Sens (%)	Sign	Spec (%)	Spec (%)	Sign
Serology	96.1	95.8	ns	62.3	66.7	ns
Rapid urease test	97.5	95.8	ns	58.7	43.8	0.017
Culture	89.7	79.2	0.016	100	100	ns
PCR total	98.9	100	<0.001	97.2	100	<0.001
Conventional PCR *cagA*	39.1	33.3	ns	100	100	ns
Conventional PCR *vacA*	90.8	75.0	0.001	97.2	100	<0.001
Conventional PCR *ureC*	95.4	91.7	ns	97.2	100	<0.001
Real-time PCR	96.6	100	<0.001	97.2	100	<0.001

yrs, years of age; Sens, sensitivity; Spec, specificity; Sign, significance level of difference (P-value), based on χ^2-test; ns, non-significant P-value (*i.e.* P>0.05); PCR, polymerase chain reaction. Diagnostic standard: see the definition under methods.

Table 6. Real-time polymerase chain reaction quantification values for different patient groups.

Groups	N	Ct ant	(95% CI)	Ct corpus	(95% CI)	Sign
Positive real-time PCR	95	30.65	(29.92-31.38)	31.52	(30.86-32.18)	0.01
High density	85	30.52	(29.72-31.31)	31.66	(30.95-32.37)	0.001
Low density	10	31.77	(30.04-33.51)	30.35	(28.68-32.03)	ns
Age >75 years	22	30.70	(29.07-32.33)	30.99	(30.01-31.98)	ns
Age ≤75 years	73	30.63	(29.79-31.47)	31.68	(30.87-32.49)	0.006

Ct, Cycle threshold value for antral/corpal biopsies; CI, confidence interval; Sign, significance level of difference (P-value), based on a paired Student's t-test; ns, non-significant P-value (*i.e.* P>0.05); PCR, polymerase chain reaction. Positive real-time PCR: all patients with positive real-time PCR where Ct-value was recorded; High Density: patients with a presumably high density of bacteria in the gastric mucosa, *i.e.* primary diagnostics; Low Density: patients with a presumably low density of bacteria in the gastric mucosa, *i.e.* having received recent anti-HP or PPI therapy.

our study showed a lower cycle threshold (Ct) value in antral biopsies than in corpal biopsies (Table 6). The absolute difference was 0.87 units, which was statistically significant. Given an optimum PCR efficiency a Ct difference of 0.87 units would indicate that antral biopsies contain about $2^{0.87}$=1.83 times higher *H. pylori* DNA concentration than corpal biopsies, and will therefore have clinical relevance for *H. pylori* diagnosis. In other words, our study shows that real-time PCR is more sensitive in antrum biopsies. However, in subgroups of patients with a presumably low bacterial density, *i.e.* patients having received recent anti-HP or PPI therapy, no difference in Ct-value was found between antrum and corpus (Table 6). This is in accordance with the established knowledge about the pathogenesis of *H. pylori*; The bacteria colonize the antrum of the stomach to avoid the acid-secreting parietal cells located in the corpus, and this mechanism is most prominent in patients with a high production of gastric acid.[24] In patients with a presumably low bacterial density, *i.e*. patients having recently gone through anti-HP or PPI treatment, the colonization pattern can be somewhat different.

The *ureC* gene was not surprisingly found to be significantly more sensitive than *vacA* and *cagA* in detecting *H. pylori* through PCR. *ureC* codes for phosphoglucosamine mutase, an enzyme essential for the survival of *H. pylori*.[7] It is present in all strains of the bacterium and has been shown to be well suited as a tracing gene.[1,14] Both *vacA* and *cagA* code for proteins that act as virulence factors. The *vacA* gene, though also present in all strains, shows considerable polymorphism,[25] which could explain it to be less sensitive than *ureC* as a molecular diagnostic marker for *H. pylori*.[10,14] We found *cagA* to be present in 37% of the *H. pylori* strains in our study, which is in agreement with the low prevalences reported in Western populations.[26,27]

There was a tendency towards better sensitivity for real-time PCR than conventional PCR, but the difference did not reach statistical significance. Real-time PCR is faster and offers several important advantages over conventional PCR; quantification and possibility for detection of mutations associated with antibiotic resistance.[1,20,28,29] Real-time PCR has therefore become the routine method for detecting *H. pylori* in our hospital.

The sensitivity and specificity of rapid urease testing in this study was 97.1% and 55.7%, respectively (Table 2). We performed as recommended a delayed reading of the Hut-Test® agar after 24 hours when initial reading (1 hour) was negative.[13] This increases sensitivity, but could also explain the low specificity in our patients, because delayed reading increases the possibility of interference from other urease positive bacteria in the gastric and oral mucosa.[1,30,31]

Sensitivity and specificity for serology was found to be 96.0% and 63.2%, respectively (Table 1). Corresponding values has been published recently in a similar populations.[18] The low specificity of serology probably reflects acid suppression or recent clearance of infection in a substantial proportion of patients (Table 4).[32]

A possible question that can be raised about the current study is the solidity of the defined diagnostic standard. To sum up, all 4 tests were in agreement in 99 of 201 patients. Three tests were in agreement in a further 63 patients: 39 patients had two tests in agreement, whereof 35 showed agreement between culture and PCR; 4 patients had to be reviewed in detail to establish their most probable *H. pylori* status. The utmost care was taken to ensure a correct labeling of *H. pylori* status, but the possibility of erratic labeling must still be acknowledged.

Conclusions

In summary, a universal gold standard assay for the diagnosis of *H. pylori* has not yet been established. The diagnosis of *H. pylori* should be carried out in light of the clinical setting, and often a combination of assays is necessary. When patients have an upper endoscopy with biopsy harvesting, PCR, especially real-time PCR, offers several advantages over culture. It is arguably less labor-intensive, less vulnerable, faster, and more sensitive. In our hand the best sensitivity was achieved using the *ureC* gene. Our routine for detection of *H. pylori* is now PCR, supplemented by culture only in patients with failed empiric eradication therapy and demand for testing of antibiotic resistance for identification and evaluation of effective *H. pylori* therapies.

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