

REVIEW ARTICLE

Detection of *Helicobacter pylori* in faeces by culture, PCR and enzyme immunoassay

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Various techniques such as culture, PCR and enzyme immunoassay have been used to detect *Helicobacter pylori* infection in human faecal specimens. Attempts to culture *H. pylori* have had limited success as the bacterium exists predominantly in a non-culturable (coccioid) form in the faeces. Several PCR protocols, differing from each other in the choice of genomic targets and primers, have been used to detect *H. pylori* infection. Substances in faeces that inhibit PCR have been removed by various pre-PCR steps such as filtration through a polypropylene membrane, biochemical separation by column chromatography and isolation of *H. pylori* with immunomagnetic beads, the former two techniques yielding results with a high degree of sensitivity and specificity. An enzyme immunoassay based on the detection of *H. pylori* antigen in faeces has become a convenient tool for the pre-treatment diagnosis of the infection. The stool antigen assay is convenient, especially for children, as it involves neither surgery nor the discomfort associated with the urea breath test. However, its applicability in monitoring eradication therapy has been controversial, as the assay can detect dead or partially degraded bacteria long after actual eradication, thus giving false positive results.

Introduction

Helicobacter pylori is a fastidious, gram-negative, flagellate bacterium known to colonise only man [1]. It is recognised as the major cause of gastritis and peptic ulcer and has been classified as a carcinogen class I [2]. Long-term carriage can lead to gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) gastric lymphoma [3]. Various tests have been developed to diagnose the infection [4]. *H. pylori* can be detected by non-invasive and invasive methods, the latter requiring endoscopy. Non-invasive testing for *H. pylori* can be done by measuring exhaled ¹³C-labelled CO₂ (known as the urea breath test, UBT), by serology and by analysing body materials such as faeces, saliva and urine [5–9]. However, positive results obtained by serology do not necessarily indicate current infection by *H. pylori*. The UBT requires an expensive instrument such as a mass spectrometer, which is not always available in routine clinical laboratories. Individuals are infected with *H. pylori* world-wide, but the prevalence is very high in economically less developed regions [10]. The presence of *H. pylori* in

faeces is compatible with a faecal–oral route of transmission, as faeces can contaminate the natural water supplies commonly used by people in poorer regions of the world. Faeces contain various materials such as diverse micro-organisms, inorganic compounds (mostly calcium and phosphates), bile salts, polysaccharides, undigested plant fibres, numerous degradative enzymes, mucus and insoluble products of the gastrointestinal tract [11, 12]. Because of ease of availability, faeces can be a convenient sample for the detection of *H. pylori*. Faecal testing is particularly appropriate for children as faeces can be obtained from them without their active collaboration, as compared with samples collected by endoscopy or for UBT. Furthermore, the patients can supply the material in the privacy of their homes. A few techniques such as bacterial culture, PCR and enzyme immunoassay have been used to detect *H. pylori* in faeces. The present review attempts to summarise our present knowledge on the detection of *H. pylori* in faeces by these techniques.

Culture

The most specific method to detect *H. pylori* is culture of the bacterium from the clinical specimen. However, very few investigators have isolated *H. pylori* from faeces [7, 13, 14]. The presence of massive numbers of

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diverse micro-organisms in faeces may make it very difficult for a fastidious bacterium such as *H. pylori* to grow. Thus, *H. pylori* differs from other easily cultivable enteric bacteria such as *Escherichia coli*, *Shigella* spp., *Salmonella* spp. or *Vibrio cholerae* [15]. The mechanism by which viable *H. pylori* can be excreted in the faeces is not properly understood. *H. pylori* colonises areas of the stomach which are not in contact with bile [16] and the bacterium is known to be sensitive to bile. As bile is present in the duodenum and the colon [17], the organism may not survive transit through the alimentary tract in association with the faeces.

In a study from the Gambia, *H. pylori* was cultured from fresh faeces of nine of 23 infants between the ages of 3 and 27 months and from the diarrhoeal stool of an adult, suggesting that the faeces of some individuals may contain viable *H. pylori* [13]. A later study showed that Gambian children were malnourished with evidence of reduced gastric acid secretion [18] and thus the Gambian children in the previous study may also have been malnourished [13]. The isolation of *H. pylori* from the faeces of these children may have been possible because *H. pylori* is released in a viable form in the faeces only under special circumstances. Children have a shorter intestinal transit time than adults. Moreover, acute *H. pylori* infection in children has been associated with decreased gastric acid secretion [18]. Investigations with ferrets have revealed that decreased acidity in the stomach may cause enhanced detection of a *Helicobacter* spp. in the faeces [19]. Therefore, transmission of *H. pylori* through the gut might have taken place in a selected group of Gambian patients who were hypochlorhydric or achlorhydric.

In another study, *H. pylori* was cultured from fresh stool specimens (< 1 h after excretion) from 12 of 25 known *H. pylori*-infected patients [7]. This indicates that *H. pylori* may not survive for a long time in the faeces. However, the authenticity of these *H. pylori* isolates has not been established, as they were not tested for oxidase activity and their identity was not established by sequence analysis of the 16S rRNA gene. Man can also be infected with non-*pylori Helicobacter* species such as *H. heilmannii*, *H. pullorum*, *H. cinaedi* and *H. fennelliae* [20]. Clinical isolates cannot be adequately classified on the basis of their shape (i.e., spiral or curved) alone. Phylogenetic relationships among bacterial species are best determined by sequence analysis of the 16S rRNA gene and ideally this technique should be used for the proper identification of clinical isolates from faecal specimens.

Recently, Parsonnet *et al.* [21] were able to cultivate *H. pylori* from 22% of faecal samples, obtained artificially by a cathartic (i.e., purgative) agent. However, they could not isolate *H. pylori* when normal stools were sampled. Faecal samples obtained artificially with a

purgative do not represent the natural conditions of faecal transit. Attempts have been made to inactivate bile acids by treating faecal suspensions from *H. pylori*-infected patients with cholestyramine, a basic anion-exchange resin that binds bile acids [14]. However, *H. pylori* could be cultured only from the faeces of 5 (26%) of 19 *H. pylori*-infected patients in this study.

Our understanding of the form and viability of *H. pylori* in the faeces is far from complete. The *H. pylori* bacterium is spiral-shaped or a curved rod when observed *in vivo* [20]. In-vitro experiments have shown that bile acids can alter the morphology of *H. pylori* to a spherical shape [16]. Therefore, it is possible that during its transit through the bile-laden, anaerobic environment of the duodenum and the colon, *H. pylori* might be converted to the spherical or the coccoid form. In older cultures the bacteria could take the coccoid form, which has been described as a viable and resting form [22]. According to one study, the amount of nucleic acid is significantly reduced in the coccoid form, indicating that this form of *H. pylori* may be the morphological manifestation of bacterial cell death [23]. However, another study found that the DNA composition was similar in two forms, suggesting that the coccoid form was probably viable [24]. A recent study demonstrated that in an anaerobic environment *H. pylori* exists predominantly in the coccoid form, but a significant proportion of bacterial cells retain viability as judged by acridine orange staining and the accumulation of polyphosphates [25]. The coccoid form of *H. pylori* has been reported to induce gastritis in BALB/c mice [26], but these results have not been reproduced in pigs [27], further illustrating the controversy as to the pathological significance of the coccoid form.

PCR

Molecular tests based upon PCR enable the specific detection of nucleic acid and have been used for the diagnosis of *H. pylori* in clinical specimens. PCR tests for *H. pylori* that use a range of genomic targets have been reviewed recently [28]. Because of its high sensitivity, PCR is suitable for diagnosis when an organism is present in low numbers, slow growing or difficult to identify. However, despite high sensitivity, the technique is susceptible to inhibition by contaminants present in the clinical specimens, thus giving false negative results. Human faeces are known to contain PCR inhibitors, which should be removed from the specimen before target DNA amplification [29]. It has been suggested that the inhibition of PCR can be overcome by dilution of the faecal suspension [30], but this may make the assay less sensitive, as fewer bacteria would be present in the diluted sample. Several procedures have been developed to purify DNA from faeces for PCR amplification.

Removal of PCR inhibitors by filtration

DNA prepared from the filtrate, after faecal suspensions were filtered through a polypropylene membrane, did not contain PCR inhibitors [31]. Primers specific for a 411-bp DNA fragment of the *ureA* gene of *H. pylori* yielded positive results in 44 of 46 *H. pylori*-infected patients determined by histology (sensitivity 95.6%) (Table 1) [31]. Faecal specimens from *H. pylori*-free patients yielded negative results (specificity 100%). With this method, it was found that the *H. pylori cagA* gene was present in 18 (60%) of 30 faecal samples [32], confirming the observation that c. 60–80% of *H. pylori* isolates carry the CagA protein, regarded as a virulence factor [33]. Filtration of faecal suspensions through various macroporous membranes other than polypropylene (e.g., nylon, polyester, polyethylene and fluorocarbon) did not remove PCR inhibitors [34]. Xantham gum, a complex polysaccharide, was retained only by the polypropylene filter, indicating that polysaccharides may act as PCR inhibitors. The mechanism of retention of polysaccharides by the polypropylene filter has not been elucidated.

Immunomagnetic separation (IMS)-based PCR

Paramagnetic beads coated with antibody to surface antigens of *H. pylori* have been used to isolate both the rod and the coccoid forms of *H. pylori* from seeded faecal suspensions [35]. Immunomagnetic separation (IMS) concentrates the bacteria from crude contaminants, thereby reducing the presence of PCR inhibitors. IMS with a monoclonal antibody to urease A has been used to isolate *H. pylori* from the faeces of Japanese dyspeptic patients with proven *H. pylori* infection [36]. The immunomagnetic bead–bacteria complex was washed to remove PCR inhibitors, treated with a lysis buffer and heated to extract DNA. A primer pair derived from the nucleotide sequence of *ureA* of *H. pylori* was used to amplify a 411 bp DNA fragment (Table 1) [36]. Thirty-five (61%) of 57 *H. pylori*-infected patients had positive faecal samples. None of the 15 *H. pylori*-negative patients had PCR-positive faecal samples (specificity 100%).

Paramagnetic beads coated with polyclonal anti-*H. pylori* antibody have been used in the pre-PCR step to isolate *H. pylori* from the faeces of children [37]. DNA was obtained by boiling the bead–bacteria complex. The PCR assay used primers to amplify the 411-bp segment of the *H. pylori ureA* gene and 41 (60%) of samples from 68 children were positive. PCR has also been used to detect *H. pylori* in normal (non-cathartic) and artificially induced (cathartic) stool specimens from 16 subjects infected with *H. pylori*. A pair of primers specific for a 139-bp product of the *H. pylori* 16S rRNA gene detected *H. pylori* DNA in 5 (31.3%) of 16 normal faeces and in 11 (68.8%) of 16 faecal specimens obtained with a cathartic agent [21].

The sensitivity offered by IMS is lower than that

obtained with other pre-PCR steps (Table 2). Although IMS is a valuable enrichment technique, it may not remove all PCR inhibitors from the faecal suspensions. *H. pylori* is known to exhibit great genetic diversity, but IMS is antigen specific and is of use only when a particular antigen can be targeted. Therefore, IMS can have only limited application as a pre-PCR step.

Biochemical purification of faecal DNA for PCR

Chromatography on a Qiagen column containing silica gel has been used to adsorb nucleic acids from the faecal cell lysates [38]. Carbohydrates and proteins are not retained by the matrix and the bound nucleic acids are eluted under low salt conditions. A pair of primers based on the nucleotide sequence of a gene encoding a species-specific 26-kDa protein antigen of *H. pylori* was used to amplify an *H. pylori* DNA fragment (c. 300 bp). The reaction was modified by a semi-nested PCR, which amplified a 209-bp fragment of *H. pylori* DNA in the faeces of 59 of 63 patients with proven infection by histology and culture (93.7% sensitivity), but in none of the 37 uninfected controls (100% specificity). This PCR assay has been used to monitor the efficiency of eradication 1 month after therapy. Of 55 patients, 41 were considered to be *H. pylori*-free and 14 still infected as judged by histology and culture. PCR was positive in 13 (92.9%) of 14 infected patients. However, PCR was still positive in 21 of 41 *H. pylori*-eradicated patients, indicating that 1 month may be too soon after therapy to monitor eradication efficiency. As PCR cannot distinguish between living and dead organisms, the persistence of *H. pylori* DNA arising from dead or partially degraded bacteria might contribute to the observed false positive results.

When faecal extracts were subjected to column chromatography and the eluates were analysed for *H. pylori* DNA by PCR, it was observed that inhibitors in the faeces were complex polysaccharides [39]. These probably originate from vegetable materials in the diet, as faeces from a subject consuming a non-vegetarian diet did not contain PCR inhibitors [40].

Acidic polysaccharides have been found to inhibit PCR [41]. Cetyltrimethyl ammonium bromide (CTAB) has been used to prepare polysaccharide-free DNA for PCR amplification [30, 42]. A pair of primers specific for a 109-bp fragment of the 16S rRNA gene of *H. pylori* yielded positive results in 28 of 31 patients with gastritis, a sensitivity of 90%. Faeces from 11 patients who had a normal gastric biopsy were not positive in the assay (specificity 100%). However, van Zwet *et al.* [43], with similar techniques, could not detect *H. pylori* DNA in the faeces of 24 *H. pylori*-infected patients with similar techniques.

A procedure in which lipid solubilisers, detergents (ionic and non-ionic), CTAB and organic solvents were used has been developed to purify faecal DNA [44].

Table 1. Conditions for different PCR-based protocols for the identification of *H. pylori* in faeces

Target gene	Nucleotide position	Primer and sequence (5'–3')	Product size (bp)	PCR conditions	Amplimer verification	Ref. no.
<i>ureA</i>	304–321	HPU1: GCCAATGGTAAATTAGTT	411	95°C for 5 min (1 cycle); 94°C for 1 min, 45°C for 1 min, 72°C for 1 min (35 cycles); 72°C for 7 min	Gel electrophoresis	[31]
	697–714	HPU2: CTCCTTAATTGTTTTTAC HPU1–HPU2		94°C for 30 s, 45°C for 90s, 72°C for 60 s (40 cycles), 72°C for 90 s (10 cycles)	Gel electrophoresis	[36]
<i>cagA</i>	2593–2612 2992–2973	AATACACCAACGCCTCCAAG TTGTTGCCGCTTTTGCTCTC	400	94°C for 4 min (1 cycle); 94°C for 1 min, 59°C for 1 min, 72°C for 1 min (35 cycles), 72°C for 10 min	RFLP of PCR products	[32]
SSA	474–496	Primer 3: TGGCGTGTCTATTGACAGCGAGC	303	98°C for 10 min (1 cycle); 92°C for 30 s, 68°C for 1 min (1 cycle); 92°C for 30 s, 68°C for 1 min (37 cycles); 72°C for 2 min (6 cycles)	Dot-blot hybridisation with a biotinylated DNA probe	[38]
	776–754	Primer 4: CCTGCTGGGCATACTTCACCATG Semi-nested PCR	209	94°C for 5 min (1 cycle); 94°C for 30 s, 68°C for 1 min, 72°C for 45 s (30 cycles), 72°C for 5 min		
16S rRNA	834–853	Hp1: CTGGAGAGACTAAGCCCTCC	110	95°C for 30 s, 55/60°C for 30 s, 72°C for 30 s (20/30/40 cycles), 72°C for 5 min	Gel electrophoresis	[42]
	744–763	Hp2: ATTACTGACGCTGATTGTGC Hp1–Hp2		94°C for 2 min, 60°C for 30 s, 72°C for 1 min (1 cycle), 94°C for 30 s, 60°C for 1 min, 72°C for 1 min (39 cycles), 72°C for 5 min	Southern blot with a digoxigenin-labelled 20-mer oligo probe	[43]
	691–710 829–809	GCGACCTGCTGGAACATTAC CGTTAGCTGCATTACTGGAGA	139	95°C for 30 s, 60°C for 1 min, 70°C for 1 min (40 cycles), 70°C for 5 min	Southern blot with a [³² P]-labelled 16-mer oligo probe	[44]
DNA 0.86 kb		EHC-U: CCCTCACGCCATCAGTCCCAAAAA EHC-L: AAGAAGTCAAAAACGCCCAAAAA	417	94°C for 45 s, 59°C for 30 s, 72°C for 45 s (30 cycles), 72°C for 10 min	Southern blot with a [³² P]-labelled 0.86-kb DNA probe	[46]

Table 2. Comparison of various pre-PCR steps for the detection of *H. pylori*

Pre-PCR step*	Target gene	Sensitivity	Specificity	Ref. no.
Removal of PCR inhibitors by a polypropylene filter	<i>ureA</i>	95.6	100	31
Isolation of <i>H. pylori</i> with immunomagnetic beads	<i>ureA</i>	61.0	100	36
DNA purification by various biochemical techniques	Species-specific antigen (26 kDa) gene [†]	93.7	100	38
	16S rRNA (bp 110) [‡]	90.0	100	42
	16S rRNA (bp 139) [§]	73.0	100	44
	0.86 kb DNA [‡]	25	90	46

*Major steps involved in the purification of DNA.

[†]DNA purified by column chromatography and concentrated over a Microcon filter.

[‡]DNA purified by CTAB.

[§]DNA isolated by the use of lipid solubilisers, ionic and non-ionic detergents.

PCR amplification was performed with a pair of primers specific for the 139-bp oligonucleotide of the *H. pylori* 16S rRNA gene. The fragment was detected in 8 of 11 patients with proven *H. pylori* infection by histology (sensitivity 73%). Subjects without infection did not produce any positive PCR results (specificity of 100%). This procedure for the isolation of DNA is lengthy and labour-intensive. Furthermore, it produces results with a lower sensitivity than those obtained by other pre-PCR steps (Table 2). Although CTAB has been used to remove faecal polysaccharides, the possibility that it can degrade DNA has also been raised [45].

Other PCR strategies

PCR has been used to identify *H. pylori* DNA indirectly in faecal samples by culture of stool specimens and then amplification of an *H. pylori* DNA fragment in the isolated bacterial cultures [7]. *H. pylori* was cultured from the faeces of 12 of 25 patients with proven infections. Primers specific for the *ureA* gene of *H. pylori* could amplify a 411-bp fragment in cultures from three patients, while DNA from two of these isolates was amplified by primers specific for the *cagA* gene.

At times, the detection rate of *H. pylori* DNA by PCR in faecal samples has been poor [46]. *Hind*III-digested DNA fragments derived from an *Eco*RI-digested 6.5-kb fragment of chromosomal *H. pylori* DNA were cloned into a plasmid. A pair of 24-base nucleotide primers, selected from the sequence data of the 0.86-kb DNA fragment, amplified a product of 417 bp from the genomic DNA of *H. pylori*. The primer pair detected *H. pylori* DNA in only 15 (25%) of 61 patients with infections proven by histology. The reason behind this poor performance has not been explained. Although several primer pairs for different genomic targets of *H. pylori* have been used, conflicting and variable results have been obtained with the same primer pair in different laboratories [28]. Therefore, it is advisable to

use at least two sets of primers that target different genes for PCR-based detection of *H. pylori* in faeces.

Stool antigen enzyme immunoassay (**Premier Platinum HpSA**)

A commercial kit based on an enzyme-linked immunosorbent assay (ELISA) that detects *H. pylori* antigen in stool specimens has been marketed recently (Premier Platinum HpSA Test, Meridian Diagnostics, USA; US patent no. 5716791). To circumvent the problem of strain variation in *H. pylori*, the kit manufacturer has selected one *H. pylori* strain, which can be found in different geographic regions and dietary groups [47]. Bacterial sonicates were prepared and the supernate obtained after centrifugation was used to immunise rabbits. The resulting polyclonal anti-*H. pylori* antibody is adsorbed to the microwells of ELISA plates and forms the basis of a capture assay. Diluted stool samples and a peroxidase-conjugated polyclonal anti-*H. pylori* antibody are added and, after incubation and routine washing, enzyme substrate is added to the wells and the resulting colour change is measured.

The stool antigen assay (HpSA) has produced promising results for the detection of *H. pylori* in faecal samples. A prospective multicentre European study evaluated the accuracy of HpSA for active *H. pylori* infection in 501 patients [48]. The results were compared to those obtained with (a) invasive tests requiring endoscopy (rapid urease test, histology, culture) and (b) the non-invasive UBT. The sensitivity and specificity of HpSA were 94.1% and 91.8%, respectively, whereas those offered by the UBT were 95.3% and 97.7%, respectively. Several studies with HpSA in various regions of the world have shown comparable sensitivities (91–98%) and specificities (83–100%) [49–57].

Most *H. pylori* infections are acquired during child-

hood or adolescence and 90% of children with duodenal ulcers are infected [58]. Although the UBT has been used to detect *H. pylori* in children, it is not ideal as some children do not co-operate to exhale $^{13}\text{CO}_2$ on request. Gastric biopsy samples can be taken from children by endoscopy, but the procedure may necessitate sedation or general anaesthesia. Therefore HpSA appears to provide an attractive method for the detection of *H. pylori* in children. In one study involving 53 children, the performance of HpSA was compared with six other diagnostic tests (UBT, culture, biopsy urease test, histology, serology and PCR) [59]. The diagnostic accuracy of HpSA was comparable (sensitivity 92.6%, specificity 100%) to the other tests. Similar detection rates with high sensitivities (85–92%) and specificities (82–98%) in paediatric populations have also been reported from a few laboratories [60–62]. These results suggest that HpSA has the potential to diagnose *H. pylori* infection in children. When this assay was used on a number of Turkish children living in Germany, it was found that the acquisition of *H. pylori* infection occurred mainly in the first 2 years of life [63].

HpSA has been used in a few studies to monitor the efficacy of eradication therapy. In a European multi-centre study, 279 of 501 patients were found to be *H. pylori* positive and 162 of them received eradication therapy [64]. Four weeks after completing therapy, patients were examined by oesophago-gastroduodenoscopy (EGD) with biopsies, UBT and HpSA. The results of EGD-based methods, used as the gold standard, showed that *H. pylori* infection in 130 of the 162 treated patients had been eradicated. HpSA was negative in 125 and UBT was negative in 129 of the 130 patients. A total of 32 patients remained *H. pylori*-infected as judged by EGD-based methods. HpSA was positive in 30 and UBT was positive in 29 of the 32 patients. These results demonstrate the sensitivity and specificity of HpSA for the accurate detection of eradication as 93.8% and 96.9%, respectively. A group of 112 Japanese peptic ulcer patients was monitored 4 weeks after the end of eradication therapy [65]. Results for *H. pylori* detection were negative in 102 and 103 patients by HpSA and UBT, respectively, indicating potential for HpSA as a means of monitoring eradication therapy. Similar promising results 4 weeks after eradication therapy have also been reported in a German study [55]. However, several other studies have questioned the efficacy of HpSA in monitoring eradication therapy. In one study involving 270 patients, 116 who were infected with *H. pylori* underwent gastroscopy (histology and rapid urease test) and UBT after treatment [53]. Stool specimens from these patients were analysed by HpSA. Two months after anti-*H. pylori* therapy, HpSA gave false positive results in 13 patients. Twelve of these 13 patients underwent a further UBT and HpSA 2–6 months after the first evaluation. Nine of the 12 patients were still HpSA positive, while 11 of 12 were UBT negative.

In another study, the efficiency of eradication therapy after 1 month was monitored by HpSA [38]. Of 55 patients, 41 were considered to be *H. pylori*-free and 14 still infected as judged by histology and culture. HpSA gave positive results for 12 (85.7%) of the 14 infected patients. However, positive results were also obtained for 13 of the 41 *H. pylori*-free patients by HpSA, indicating that 1 month may be too soon after treatment to monitor eradication efficiency. False positive results are less common with specimens obtained 8 and 12 weeks after the end of the treatment [66].

In another study, HpSA displayed high sensitivity (89.5%) for the diagnosis of *H. pylori* infection, but low specificity (77.8%) [67]. The specificity increased to 83.3%, with no change in sensitivity, when the investigators used a higher absorbance cut-off level than that recommended by the manufacturer. When used to monitor eradication therapy, the sensitivity of the test was 70.4% and 50% at 6 weeks and 6 months after treatment, respectively. These results indicate that HpSA may not reliably predict the outcome of the eradication treatment. Factors such as the persistence of *H. pylori* coccoid forms or degenerating forms of dead bacteria, or cross-reactivity with other strains of *Helicobacter* that colonise humans may contribute to the high rate of false positive results.

Limitations and advantages of HpSA

The HpSA test is qualitative and no quantitative interpretation should be attempted. The results should be studied in conjunction with other diagnostic procedures. The test should not be done on patients who have taken medications containing antimicrobial agents, proton pump inhibitors and bismuth preparations, as these substances may suppress the growth of *H. pylori* and cause false-negative results. The test has not been validated for use on watery diarrhoeal stools. According to the manufacturer, the assay has been found to be specific for *H. pylori* and did not give false positive results when stool samples were spiked with a number of other enteric micro-organisms. However, it has not been tested against non-*pylori* urease-positive *Helicobacter* spp. other than *H. pylori* that are found in the human stomach. What the test actually detects in the faeces has not been elucidated. It is possible that it detects both living or dead *H. pylori*, or both, or partially digested bacteria from the stomach.

The test is suitable for pre-treatment diagnosis as well as for epidemiological studies. The colour developed by the test can even be monitored visually (sensitivity 94%, specificity 99%) [68]. Many patients infected with *H. pylori* are treated by general practitioners and, as HpSA is easy to perform and patient friendly, it is suitable for side-room use. It is cheaper than the non-invasive UBT and its cost is similar to those of serological tests [69].

Table 3. Advantages and limitations of diagnostic tests for the detection of *H. pylori* in the faeces

Diagnostic test	Advantages	Limitations
Culture	Maximum specificity and accuracy in identification Easily set up in medical laboratories Permits strain typing Antimicrobial susceptibility testing possible	Very difficult to culture from the faeces Limited information on the form and viability of <i>H. pylori</i> in the faeces Delayed result
PCR	High sensitivity and specificity Low numbers of organisms detectable Quick results possible Detection of <i>cagA</i> -positive strains possible	Removal of PCR inhibitors from the faecal samples required Susceptible to contamination Distinction between live and dead organisms not possible Persistence of <i>H. pylori</i> DNA in the faeces may give false positive results in monitoring early eradication therapy Not suitable in the routine clinical setting
Stool antigen enzyme immunoassay (HpSA)	Patient friendly Convenient for pre-treatment diagnosis as high sensitivity and specificity are attainable Excellent epidemiological tool Quick results possible Easily set up in medical laboratories Results can be monitored visually	Monitoring eradication therapy remains controversial The assay may detect both living and dead, or partially degraded bacteria Not suitable for identifying <i>cagA</i> -positive <i>H. pylori</i> infection

Stool antigen enzyme immunoassay (FemtoLab *H. pylori*)

Another stool enzyme immunoassay kit, manufactured by FemtoLab *H. pylori* of Germany, uses monoclonal antibodies against *H. pylori* antigens. The assay showed sensitivity and specificity of 98% and 96.7%, respectively, in diagnosing *H. pylori* infection [66]. It produced a specificity for eradication therapy of 96.9% with specimens obtained 4 weeks after the treatment. This value increased to 100% with stool samples collected 6 and 8 weeks after the end of treatment, as no false positive results were obtained. However, more multicentre-based studies are needed to establish the acceptability of this kit.

Conclusions

Non-invasive tests on faecal samples can play an important role in the diagnosis of *H. pylori* infections. The advantages and limitations of various tests for the clinical identification of *H. pylori* in the faeces have been summarised in Table 3. Culture should be regarded as the most specific test for the presence of *H. pylori*. The major advantage of culture as a diagnostic tool is that the isolation of the organism can assist in the choice of antibiotic treatment. However, the success rate for the isolation of *H. pylori* from faeces has been rather poor, and little information is available on the form and viability of *H. pylori* in the faeces. PCR, a powerful method known for its high sensitivity, can detect low numbers of *H. pylori* present in specimens such as the faeces, but faeces are known to contain PCR inhibitors and this limits the direct application of PCR to faecal samples. Among various methods developed to remove PCR inhibitors, filtration of the faecal suspensions through a polypropylene

membrane appears to be effective. However, more multicentre studies are needed before it can be recommended. Although PCR has been used to follow up eradication therapy, the persistence of *H. pylori* DNA yields false positive results and restricts its use for early monitoring of treatment efficacy. PCR requires specialised laboratory facilities for its execution and is not generally available as a routine diagnostic tool. The stool antigen assay (HpSA) is a convenient pre-treatment diagnostic test. Results can be monitored visually, thus making it very useful for a general practitioner. However, there are controversies over its use in eradication therapy, especially at an early stage after treatment. Therefore, a simple and reliable test that can be used to monitor eradication treatment, for both short and long periods, is still required.

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