

12. INTERPRETATION OF THE TEST RESULTS

12.1. POSITIVE CONTROL AND NEGATIVE CONTROL

As detailed in Section 11.1 (Preparation of Controls) at least one negative and one positive control must be included in each assay run.

The following quality criteria should be met:

Positive Control OD_{450 / 620 to 650nm} > 1.00 (OD_{450nm} > 1.04)
Negative Control OD_{450 / 620 to 650nm} < 0.10 (OD_{450nm} < 0.14)

12.2. SPECIMENS

Test results are interpreted as follows:

Dual wavelength (450/620 to 650nm)

Specimens with absorbance values ≥ 0.150 are positive.

Specimens with absorbance values < 0.150 are negative.

Single wavelength (450nm)

If it is not possible to use a reference wavelength between 620 and 650nm on the microplate reader, the cut-off is as follows:

Specimens with absorbance values ≥ 0.190 are positive.

Specimens with absorbance values < 0.190 are negative.

A positive test result indicates the presence of *H. pylori* antigens. A negative result indicates the absence of *H. pylori* antigens or a concentration of antigens below the detection limit.

If, after the addition of substrate, the content of a microwell turns dark blue and forms a blue-black precipitate, the specimen should be interpreted as positive.

13. PERFORMANCE LIMITATIONS

13.1. Amplified IDEIA Hp StAR is a qualitative test and no quantitative interpretation should be made. Test results should be interpreted by the clinician in conjunction with clinical findings and/or other diagnostic procedures.

13.2. Antibiotics, proton pump inhibitors and bismuth preparations are known to suppress growth of *H. pylori*. Stool sampling must be performed not earlier than 2 weeks after termination of ingestion of proton pump inhibitors and bismuth preparations and 4 weeks after termination of ingestion of antibiotics, respectively.

13.3. A negative result does not exclude the possibility of *H. pylori* infection in the patient. Failure to detect *H. pylori* may be a result of factors such as improper sampling or handling of the specimen.

13.4. A positive test result alone does not justify an indication for eradication therapy. Other methods may be necessary to confirm *H. pylori* infection. Differential diagnosis with invasive endoscopic methods might be indicated in order to examine the presence of any other complicating conditions, eg ulcer, autoimmune gastritis and malignancies.

13.5. A test result within 0.020 absorbance units around the cut-off value should be interpreted with caution.

14. EXPECTED VALUES

Expected values depend on geographic location and type of population studied. The rate of positive test results may vary due to the type of test employed and the method of specimen collection and handling.

Epidemiological studies have shown that the infection by *H. pylori* is prevalent throughout the world. In Europe and North America 25-50% of the population carries *H. pylori*. Even higher prevalence rates of 70-90% have been reported for Asia, Africa and South America^{1,11}.

The frequency of *H. pylori* infection has been shown to correlate with age, ethnic background, socioeconomic class and the general health environment eg the prevalence of infection in the United States increases with age at approximately 1% per year¹².

15. SPECIFIC PERFORMANCE CHARACTERISTICS

15.1. CLINICAL STUDIES

Study 1: Primary diagnosis in adult patients

Amplified IDEIA Hp StAR test was evaluated on 356 patients (201 female, 155 male, age range 18 - 82 years) from 10 centers in Germany undergoing endoscopy because of abdominal pain and dyspepsia. Stool testing was performed in independent laboratories in a blinded fashion.

The patients had a variety of gastric pathologies noted, including: mild gastritis (n=61), chemical toxic gastritis (n=98), *H. pylori* associated gastritis (n=144), antral erosions (n=11), atrophic gastritis (n=2), gastric ulcer (n=5), duodenal ulcer (n=3), adenocarcinoma (n=2), submucous tumor (n=1) Schatzki's Ring (n=1), Crohn gastritis (n=1), asymptomatic (n=27).

Amplified IDEIA Hp StAR test results were compared to the diagnosis of *H. pylori* infection as judged by histology. Amplified IDEIA™ Hp StAR™ showed a sensitivity of 95.3% and a specificity of 97.1%. Confidence intervals (CI) were calculated using the exact binomial method. Results are shown in table 1.

Table 1: Primary diagnosis in adult patients using Amplified IDEIA Hp StAR and the reference test histology

Hp StAR	Histology		Sensitivity ± 95% CI
	+	-	
+	141	6	97.1% (202/208)
-	7	202	± 95% 93.8 - 98.9%

Study 2: Primary diagnosis in pediatric patients

Amplified IDEIA Hp StAR assay was tested in a study performed with faecal samples from children undergoing endoscopy because of abdominal pain and/or other intestinal disorders. 239 children (124 male, 115 female, age range 6 months to 18 years) from three pediatric gastroenterology centers in Europe were included.

As reference tests histology and culture were used. The patient was defined *H. pylori* positive if histology and/or culture were positive and *H. pylori* negative if both tests were negative. Amplified IDEIA Hp StAR showed a sensitivity of 98.6% and a specificity of 99.4%. Confidence intervals (CI) were calculated using the exact binomial method.

Table 2: Primary diagnosis in pediatric patients using Amplified IDEIA Hp StAR and the reference tests histology/culture

Hp StAR	Histology Culture		Sensitivity ± 95% CI
	+	-	
+	70	1	99.4% (167/168)
-	1	167	± 95% 96.7 - 100%

Study 3: Monitoring the response to eradication therapy in paediatric patients

40 *H. pylori* infected children (age 3 to 15 years) with recurrent abdominal pain were recruited in two pediatric gastroenterology centers. *H. pylori* infection was shown by urea breath test and serology. All 40 faecal samples were identified positive by Amplified IDEIA Hp StAR. Eradication was performed by triple therapy for seven days. Eradication control was performed by urea breath test four weeks after therapy. Amplified IDEIA Hp StAR showed a sensitivity of 100% and a specificity of 96.9%. Confidence intervals (CI) were calculated using the exact binomial method. Results are shown in table 3.

Table 3: Performance of Amplified IDEIA Hp StAR relative to UBT for monitoring response to eradication therapy in paediatric patients.

Hp StAR	Urea breath test		Sensitivity ± 95% CI
	+	-	
+	8	1	96.9% (31/32)
-	0	31	± 95% 83.8 - 99.9%

Study 4: Monitoring the response to eradication therapy in adult patients

Stool samples were collected from 93 patients in the North East of Spain (64 male and 29 female, age range 21 - 80 years) who had undergone eradication therapy for confirmed *H. pylori* infection. Patients were instructed to obtain a faecal sample the same day that the UBT for controlling eradication therapy was performed (at least 4 weeks post completion of therapy). Samples were immediately frozen and kept at -80°C then thawed and tested with Amplified IDEIA Hp StAR.

Table 4 shows the results obtained with Amplified IDEIA Hp StAR when compared with the reference method (UBT). Amplified IDEIA Hp StAR demonstrated sensitivity and specificity of 80% (8/10) and 97.6% (81/83) respectively and an overall correlation of 95.7% (89/93) relative to UBT.

Table 4: Performance of Amplified IDEIA Hp StAR relative to UBT for monitoring response to eradication therapy in adult patients

Hp StAR	Urea breath test		Sensitivity ± 95% CI
	+	-	
+	8	2	97.6% (81/83)
-	2	81	± 95% CI 91.6 - 99.7%

15.2. REPRODUCIBILITY

Intra-assay and inter-assay variations were determined by testing weak positive (n=2), medium positive (n=2) and strong positive (n=2) as well as negative samples (n=2). Reproducibility testing was performed in three independent laboratories in Europe. Each sample was tested in 10 wells at each site. Intra-assay and inter-assay coefficients of variations (CV) were calculated and are presented below. Ranges for OD-values and intra- and inter-assay variances are given for the different stool samples tested.

Table 5: Intra- and inter-assay variation of Amplified IDEIA Hp StAR

	Negative samples	Weak positive samples	Medium positive samples	Strong positive samples
OD _{450/630nm}	0.024 - 0.070	0.495-0.897	1.306 - 2.656	3.00 - 3.776
Intra-assay CV	5.9 - 17.4%	2.7 - 10.1%	2.1 - 4.0%	1.1 - 3.1%
Inter-assay CV	41.2 - 46.1%	23.0 - 25.8%	24.1 - 25.4%	8.1 - 10.2%

16. CROSS REACTIVITY

Amplified IDEIA Hp StAR stool assay is highly specific for antigens from *H. pylori*. For each strain a concentration of ≥ 1×10⁸ organisms/ml in sample buffer was tested. No cross reactivity was observed when testing the microorganisms listed below. In contrast *H. pylori* gave a positive test result.

Acinetobacter lwoffii	Providencia stuartii
Aeromonas hydrophila anaerogenes	Pseudomonas aeruginosa
Aeromonas hydrophila hydrophila	Pseudomonas fluorescens
Campylobacter fetus	Pseudomonas putida
Campylobacter jejuni	Salmonella agona
Citrobacter freundii	Salmonella choleraesuis
Enterobacter cloacae	Salmonella infantis
Enterococcus faecalis	Salmonella ohio
Enterococcus faecium	Salmonella typhimurium
Escherichia coli	Serratia proteamaculans
Escherichia hermannii	Shigella flexneri
Lactococcus lactis	Shigella sonnei
Listeria innocua	Staphylococcus aureus
Proteus mirabilis	Streptococcus agalactiae
Proteus vulgaris	Streptococcus dysgalactiae

17. REFERENCES/LITERATURE

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