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# REVIEW – DIAGNOSTIC OF *HELICOBACTER PYLORI* INFECTION

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**Abstract:** This past year, only a few articles concerning *Helicobacter pylori* endoscopic diagnosis were published but one aimed to develop a computer-aided diagnosis system based on linked color imaging. A meta-analysis also allowed to generate an algorithm useful to discriminate between non-infected and post-eradication cases. Concerning invasive tests, the best time to read the rapid urease test (RUT) was established, and the possibility of using leftover tissue from the RUT to culture the bacterium was also validated. Molecular biology was used on gastric biopsies to improve *H. pylori* detection, for example a droplet digital polymerase chain reaction managed to detect 'occult' bacteria. Concerning non-invasive tests, the urea breath test was shown to be interesting in case of upper-gastrointestinal bleeding to improve *H. pylori* detection. Different kits to detect *H. pylori* antigen in stools were evaluated. A study to develop an accurate method for predicting true *H. pylori*-uninfected patients was performed using logistic regression analysis with results from non-invasive parameters.

**Keywords:** Endoscopy, Gastric biopsy, New generation sequencing, Rapid urease test, Serology, Stool antigen test, Urea breath test.

## ENDOSCOPIC DIAGNOSIS

A meta-analysis of eight published studies focusing on endoscopic images of *Helicobacter pylori* infection and application of artificial intelligence for the prediction of *H. pylori* infection was published this year. The authors elaborated an algorithm which discriminated between the non-infected and the post-eradication cases in 82% of the 385 patients with *H. pylori* infection and the 1,334 controls[1]. This artificial intelligence algorithm could be useful as an additional tool for the prediction of *H. pylori* infection during endoscopic procedures.

Four studies focused on endoscopic techniques used to diagnose *H. pylori*. They were mainly conducted in Asia. A prospective and multicenter Chinese study aimed to determine whether the Kyoto classification-based conventional endoscopic features are effective in determining past and current *H. pylori* status[2]. Furthermore, they aimed to clarify the usefulness of the following features: "unclear atrophy boundary" (UAB) and "reappearance of regular arrangement of collecting venules" (RAC reappearance) in atrophic mucosa. This study provides evidence of the clinical accuracy and robustness of the Kyoto classification of gastritis

for the diagnosis of *H. pylori*. In addition, UAB and RAC reappearance can be considered as two new indicators of past *H. pylori* infection, potentially useful for gastric cancer screening. An innovative gastric cancer screening program was designed 1) to develop a computer-aided diagnosis (CAD) system based on linked color imaging (LCI) and 2) to classify the status of *H. pylori* infection of patients into three categories: uninfected, currently infected, and post-eradication. The LCI-CAD system had comparable diagnostic accuracy to experienced endoscopists to detect uninfected cases (91.2%), infected cases (79.4%) and post-eradication status (78.1%). The authors extrapolate that LCI-CAD may facilitate risk stratification of examinees and improve gastric cancer detection[3].

A clinical trial was carried out to evaluate whether LCI could improve the detection of *H. pylori* gastritis. One hundred patients underwent endoscopy using white light imaging (WLI) and LCI. Videos were recorded and analyzed by experts. The accuracy and sensitivity of LCI for *H. pylori* infection diagnosis were significantly higher than those of WLI, but no difference was shown to detect metaplastic gastritis between these two techniques[4].

## INVASIVE TESTS

### Rapid Urease Test

The rapid urease test (RUT) is still used by gastroenterologists to detect *H. pylori*. Two studies[5,6] were published this past year on the subject. One[5] aimed to find the best reading time combined with the best accuracy of RUT. The authors followed the RUT results of 150 patients at different times (5 min, 10 min, 20 min, 1 h, 2 h, 6 h, 12 h, 24 h) and compared them to the histology results without immunohistochemistry. RUT accuracy increased with time and the best moment to read RUT proved to be 12 h after the test performance[5]. A prospective study[6] compared the diagnostic performance of a "sweeping method" for *H. pylori* detection with the conventional biopsy sampling method in atrophic gastric conditions in 279 patients. Gastric mucosa samples from both the antrum and the corpus were swabbed, and then, the swabs were placed into the sample insertion well of the CLO kit. *H. pylori*-positive rates were as follows: sweeping method, 68.8%; conventional method, 50.5%; histology, 64.2%; and PCR, 63.1%. Sensitivity and accuracy of this sweeping method, which allows more *H. pylori* to be absorbed from the gastric mucus, were better than those obtained with conventional methods[6].

### Culture

A study performed by Gong et al[7] investigated whether *H. pylori* can be cultured from tissue samples used for the RUT. During endoscopy, two biopsy specimens, one each from the gastric antrum and the corpus, were obtained and placed into a commercially available RUT kit. After the detection of urease activity, *H. pylori* was cultured using leftover tissue from the RUT. Eighty-one *H. pylori* strains were isolated from 141 specimens with positive RUT results (57.4%), as well as 14 strains from 33 specimens with negative RUT results (42.4%). The median interval between tissue acquisition and inoculation onto the isolation media was 3.6 hours in the cases with successful culture, compared to 23.5 hours in the cases with failed culture. A RUT kit can be used as a transport medium for *H. pylori*, and this medium is most efficient when used within 4 hours of the test[7].

A study was carried out to describe the gastric distribution, density, and diversity of *H. pylori* in three stomachs collected after sleeve gastrectomy in order to determine the number and location of biopsies needed to detect the presence and resistance of *H. pylori* using culture and real-time PCR (RT-PCR) on multiple gastric sites. Diversity of *H. pylori* strains was studied using antibiotic susceptibility testing (AST), random amplified polymorphism DNA (RAPD) typing, and *cag*A gene detection on single-colony isolates. *H. pylori* was detected in nearly all the different analyzed sites (354/365 biopsies). The three stomachs were almost exclusively infected by an antibiotic-susceptible strain. One clarithromycin-resistant isolate was detected in one biopsy from each of two stomachs (1/44 and 1/49 isolates), while in the third

one, eight metronidazole-resistant isolates (8/96 isolates) were detected in the same anatomical area. DNA typing showed infection with *cag*A-negative strains for one patient, *cag*A-positive strains for a second patient and the third patient was infected with two different strains of distinct *cag*A genotypes. Infection with *H. pylori* is known to spread throughout the entire stomach surface, but the possibility of a minor sub-population of antibiotic-resistant clones, undetectable in routine practice, still exists[8].

Concerning *Helicobacter suis*, an interesting recent study[9] highlighted its possible implication in human gastric disease: the authors were able to successfully culture *H. suis in vitro*, directly from human stomach samples of three patients with gastric diseases, following the use of a low-pH medium for transport of the gastric biopsies[9].

## Molecular Methods

### *PCR*

Deng et al[10] were able to improve the RT-PCR method by designing two pairs of primers and probes targeting the *glm*M gene to quantitatively detect *H. pylori* and identify its virulence genes, *vac*As1, *vac*Am1, *cag*A and *bab*A2. They used this method on 141 gastric biopsies to detect *H. pylori* in patients and analyzed the pertinent information within the data. Their RT-PCR gave better results than RUT to detect *H. pylori*. They found that more than half of the infected patients had a strain containing virulence factors and the *vac*As1 genotype was the most frequent in their Chinese cohort. It was associated with chronic superficial gastritis, peptic ulcer, and gastric erosions.

Droplet digital polymerase chain reaction (ddPCR) is known to be more sensitive than other PCR methods. A Spanish team evaluated the ability of ddPCR to detect *H. pylori* infection in patients initially diagnosed as negative by conventional tests (Histology, RUT and UBT). Surprisingly, ddPCR targeting three *H. pylori* specific genes detected so-called 'occult' *H. pylori* in a significant proportion (36%) of 158 dyspeptic patients initially diagnosed as negative, but only two of them exhibited a chronic active gastritis. The quantification indicated a low bacterial load[11]. Such results need confirmation.

### *Next generation sequencing (NGS)*

Few studies were based on a NGS strategy. Among them, Egli et al[12] compared the diagnostic performance of a 23S rDNA quantitative PCR (qPCR) and an in-house developed *gyr*A qPCR followed by Sanger sequencing with a commercial hybridization probe assay (for 23S rDNA and *gyrA*), the Genotype HelicoDR assay (Hain Lifesciences GmbH, Nehren, Germany), using 142 gastric biopsies. They also compared the same two qPCRs with whole-genome sequencing (WGS) using Illumina platform technology and phenotypic characterization of clarithromycin and levofloxacin resistance using cultured isolates. The sensitivity of both qPCRs was 100% compared to the Genotype HelicoDR assay. The results showed a good agreement between molecular tests, especially between qPCR (including Sanger sequencing) and WGS. Discrepancies, concerning mutated or wild type *H. pylori* positive gastric biopsies, were observed between Sanger sequencing of the *gyr*A gene and the Genotype HelicoDR assay. The authors concluded that, due to high sequence variations in the *gyr*A gene, the two qPCRs followed by Sanger sequencing of the *gyr*A gene are currently the best and fastest commercially available molecular methods. WGS strongly supports the results of both qPCR using cultured isolates. However, the abundance of human DNA might reduce its sensitivity for the direct detection of *H. pylori* in gastric biopsies[12].

WGS was also used in the Democratic Republic of Congo to screen the occurrence of genotypes encoding antimicrobial resistance (AMR) in *H. pylori*. The authors reported mutations often present in Western and Asian populations and several putative AMR-related new genotypes in the *pbp*1A (e.g., T558S, F366L) related to amoxicillin resistance, *gyr*A (e.g., A92T, A129T) and *gyr*B (e.g., R579C) associated with levofloxacin resistance *in vitro* in the majority of the cases, and *rdx*A (e.g., R131_K166del) genes known to be related to metronidazole resistance[13].

## NON-INVASIVE TESTS

### Urea Breath Test

One study investigated the sensitivity and the specificity of a $^{13}$C-urea breath test (UBT), Heliforce kit (Beijing Richen-Force Science & Technology Co. Ltd., Beijing, China), in the diagnosis of *H. pylori* and in post-eradication monitoring. The $^{13}$C-UBT results were compared to endoscopy-based methods and a stool antigen test (SAT) (Abon Biopharm Hangzhou Co. Ltd., Hanghazue, China). Thirty patients were tested for *H. pylori* using $^{13}$C-UBT and SAT. Compared to endoscopy, sensitivity was 94.1% for the $^{13}$C-UBT, and 76.5% for the SAT. The accuracy of the $^{13}$C-UBT was 86.7% compared to 76.7% for the SAT. Moreover, the status 6 weeks post-*H. pylori* eradication treatment was assessed with both non-invasive tests on 30 other patients. Both tests showed a comparable outcome in assessing the success of the eradication treatment: successful eradication was observed in approximately 77% of patients using *H. pylori* SAT, while it was approximately 67% using the $^{13}$C-UBT. However, globally, the $^{13}$C-UBT was found to be more sensitive and accurate than the SAT[14].

Ojetti et al[15] used a UBT-Kit (Richen Europe, Milan, Italy) on 87 patients, admitted to an emergency department for upper-gastrointestinal bleeding (UGIB), melena or hematemesis. Compared to biopsy results (n=27), a positive predictive value (PPV) of 71% and a negative predictive value (NPV) of 80% were obtained for the UBT. The authors underlined that only 27 of the 87 patients with UGIB underwent a biopsy, meaning that if UBT had not been performed, 21 *H. pylori* diagnoses would have been missed. UBT testing of patients directly in the emergency department also resulted in a shorter length of stay and could be recommended for an etiologic diagnosis and appropriate treatment for UGIB[15].

Using UBT, Muzikami et al[16] in Japan showed that acotiamide (Acofide® tablet), a treatment for functional dyspepsia, did not have an impact on *H. pylori* diagnosis and that the treatment does not have to be interrupted to detect *H. pylori* using UBT.

Beresniak et al[17] compared the cost-effectiveness of the three main strategies used for the management of patients with dyspepsia in Spain: the "Test and Treat" (T&T) strategy including the UBT *vs.* symptomatic treatment *vs.* upper gastrointestinal endoscopy. Their results indicated that the T&T strategy was beneficial and was also the most cost-effective[17].

### Stool antigen test

A Taiwanese study carried out by Fang et al. compared the accuracy of a rapid immunochromatography test (ICT), the Vstrip® HpSA, with the US FDA-approved ImmunoCard STAT!® HpSA and Premier Platinum HpSA® PLUS, using UBT as the gold standard on 347 adults. The Vstrip® HpSA showed a sensitivity of 91%, a specificity of 97% and an accuracy of 95.7%. It was as efficient as the two other HpSAs and therefore can be used as a self-test as an *in vitro* diagnostic tool or for large-scale screening for *H. pylori* infection[18].

In France, Benejat et al[19] evaluated retrospectively the RIDA®QUICK Helicobacter and RIDASCREEN® Helicobacter kits (R-Biopharm, Darmstadt, Germany) in detecting *H. pylori* antigens in 38 stool samples. Results were compared to a RT PCR performed on gastric biopsies from the same patients. Both kits showed good performances in terms of specificity (100%) and sensitivity (92.1% for RIDASCREEN™ and 89.5% for RIDA™QUICK) and can be included in the armamentarium of diagnostic tests for *H. pylori* infection.

Another study[20] determined the performance of two new stool-based enzyme immunoassays, *H. PYLORI* QUIK CHEK™ and *H. PYLORI* CHEK™ (TechLab Inc., Blacksburg, VA, USA). SAT results were compared to histological analysis and RUT on biopsies. Their sensitivity and specificity were 92% and 91%, respectively, for the *H. PYLORI* QUIK CHEK™ and 91% and 100%, respectively, for the *H. PYLORI* CHEK™. No significant cross-reactivity against other gut pathogens was observed. Both assays demonstrated an excellent clinical performance compared to the composite reference method[20].

The performance of the SAT, CerTest *H. pylori* Blister Test (CerTest Biotec S/L, Zarogosa, Spain) was evaluated in China on an elderly male cohort of more than 300 participants, using UBT as a reference standard. The test achieved a high accuracy (91.5%) with a high specificity

(97.6%) but suboptimal sensitivity (68.7%). Constipation was associated with decreased sensitivity, while colorectal polyps were associated with increased sensitivity. Because of the low sensitivity, the authors suggested that caution should be taken when using this test on elderly patients with constipation[21].

## Molecular tests

Different methods to improve the detection of *H. pylori* DNA in human stools were tested on 100 samples in Italy. The authors developed and evaluated two TaqMan-based RT-PCR qualitative assays detecting *ure*C (*glm*M) and *cag*A of *H. pylori* on DNA extracted by three procedures. Their findings showed that the bead-beating step prior to DNA extraction, classically used for intestinal parasite infections, can reach a sensitivity of up to 94% for *H. pylori* detection in stools[22].

## Serology

An American study[23], using *H. pylori* multiplex serology and a fluorescent bead-based suspension array including a set of 13 *H. pylori* antigens, determined the sensitivity of seropositivity to *H. pylori* proteins to discriminate between active *vs.* past *H. pylori* infection, in comparison to UBT in 92 adults. Active infection was confirmed with a specificity of 91% and a sensitivity from 75 to 100% for antibodies against *H. pylori* proteins VacA, GroEl, HcpC, and HP1564. Positivity to a combination of these proteins resulted in a specificity of 90% and a sensitivity of 100%. They concluded that, in populations with generally low rates of *H. pylori* eradication therapy like in the US, *H. pylori* multiplex serology may be applicable to approximate the prevalence of active *H. pylori* infection in large epidemiological cohorts[23].

In Finland Mäki et al[24] validated the clinical performance of the *H. pylori* IgG ELISA of the New-Generation GastroPanel® (Biohit Oyj, Helsinki, Finland) in the diagnosis of biopsy-confirmed *H. pylori* infection. The two test versions of the GastroPanel® were used on more than 100 patients. *H. pylori* IgG ELISA diagnosed *H. pylori* infection in biopsy-confirmed patients with a sensitivity of 92.3% and a specificity of 88.6%[24].

A study[25] to develop an accurate method for predicting true *H. pylori*-uninfected patients was performed using logistic regression analysis with results from non-invasive parameters without the need for endoscopic examination. *H. pylori* serology (LIA method Eiken Chemical Co., Ltd., Tokyo, Japan), SAT and endoscopy were performed on 684 subjects with no history of *H. pylori* eradication. A formula using age, *H. pylori* antibody, pepsinogen I, and pepsinogen II levels was determined to improve detection of true uninfected individuals (sensitivity: 93.2%, specificity: 88.5) for whom the risk of gastric cancer differs markedly from true infected individuals.

A Japanese study[26] evaluated the accuracy of the serum antibody test for *H. pylori* infection for the mass screening of junior high school students: 410 students underwent both SAT and serum antibody test. The authors concluded that the accuracy of the serum antibody test may be sufficient for practical use in children 13-14 years old.

One study[27] evaluated a newly developed latex aggregation turbidity assay (*H. pylori*-latex 'SEIKEN', Denka Seiken, Tokyo, Japan) and a conventional ELISA (Eiken Chemical Co., Ltd., Tokyo, Japan), both containing Japanese *H. pylori* antigens, using 1,797 sera from seven Asian countries *vs.* culture and histology on gastric biopsies. The conclusion was that the two tests were applicable in all countries but new kits using regional *H. pylori* strains could be recommended in some countries.

Another study[28] reviewed *H. pylori* diagnostic techniques within a health system (2,560 cases) over a 12-year period. *H. pylori* IgG serology demonstrated a higher sensitivity (94%) than UBT (64%) and SAT (61%). The serological test showed a lower cancellation rate by patients, proving its advantage in accessibility to care. For the authors, although *H. pylori* serology has a slightly lower specificity than other non-invasive tests, its superior sensitivity and NPV in the study population support its use as a non-invasive test to rule out *H. pylori* infection.

## DIAGNOSTIC IN THE ENVIRONMENT

Concerning the presence of *Helicobacter* spp. in the environment, a study[29] focused on the presence of potentially pathogenic *Helicobacter* spp. in treated wastewater intended for irrigation in Spain. A NGS approach was used associated with a culture approach. Thanks to the wastewater microbiome analysis, *Helicobacter* genus was detected in seven samples out of sixteen, meaning that species, such as *H. pylori*, *Helicobacter hepaticus*, *Helicobacter pullorum* and *H. suis* can be present in wastewater samples, even after disinfection treatment.

### Conflict of interest

The authors declare that they have no conflict of interest.

### REFERENCES


1. Bang CS, Lee JJ, Baik GH. Artificial intelligence for the prediction of *Helicobacter pylori* infection in endoscopic Images: systematic review and meta-analysis of diagnostic test accuracy. J Med Internet Res 2020; 22: e21983.
2. Zhao J, Xu S, Gao Y, Lei Y, Zou B, Zhou M, Chang D, Dong L, Qinet B. Accuracy of Endoscopic diagnosis of *Helicobacter pylori* based on the Kyoto classification of gastritis: A multicenter study. Front Oncol 2020; 10: 599218.
3. Nakashima H, Kawahira H, Kawachi H, Sakaki N. Endoscopic three-categorical diagnosis of *Helicobacter pylori* infection using linked color imaging and deep learning: a single-center prospective study (with video). Gastric Cancer 2020; 23: 1033-1040.
4. Lee SP, Lee J, Kae SH, Jang HJ, Koh DH, Jung JH, Byeon SJ. The role of linked color imaging in endoscopic diagnosis of *Helicobacter pylori* associated gastritis. Scand J Gastroenterol 2020; 55: 1114-1120.
5. Eslaminejad A, Marashian SM, Aboutorabi M, Sadr M, Agah S. Determination of optimal time for reading of rapid urease test diagnosis of *Helicobacter pylori*. Gastroenterol Hepatol Bed Bench 2020; 13: 232-237.
6. Noh C-K, Lee GH, Park JW, Roh J, Han JH, Lee E, Park B, Lim SG, Shin SJ, Cheong JY, Kim JH, Lee KM. Diagnostic accuracy of "sweeping" method compared to conventional sampling in rapid urease test for *Helicobacter pylori* detection in atrophic mucosa. Sci Rep 2020; 10: 18483.
7. Gong EJ, Ahn JY, Jung DK, Lee SM, Pih GY, Kim GH, Na HK, Lee JH, Jung HY, Kim JM. Isolation of *Helicobacter pylori* using leftover tissue in the rapid urease test kit. Helicobacter 2020; 25: e12733.
8. Pichon M, Tran CT, Motillon G, Debiais C, Gautier S, Aballea M, Cremniter J, Vasseur P, Tougeron D, Garcia M, Garnier M, Bodet C, Faure JP, Burucoa C. Where to biopsy to detect *Helicobacter pylori* and how many biopsies are needed to detect antibiotic resistance in a human stomach. J Clin Med 2020; 9: 2812.
9. Rimbara E, Suzuki M, Matsui H, Nakamura M, Morimoto M, Sasakawa C, Masuda H, Nomura S, Osaki T, Nagata N, Shibayama K, Tokunaga K. Isolation and characterization of Helicobacter suis from human stomach. Proc Natl Acad Sci U S A 2021; 118: e2026337118.
10. Deng L, He XY, Tang B, Xiang Y, Yue JJ. An improved quantitative real-time polymerase chain reaction technology for *Helicobacter pylori* detection in stomach tissue and its application value in clinical precision testing. BMC Biotechnol 2020; 20: 33.
11. Ramírez-Lázaro MJ, Lario S, Quílez ME, Montserrat A, Bella MR, Junquera F, García-Martínez L, Casalots À, Parra T, Calvet X. Droplet digital PCR detects low-density infection in a significant proportion of *Helicobacter pylori*-negative gastric biopsies of dyspeptic patients. Clin Transl Gastroenterol 2020; 11: e00184.
12. Egli K, Wagner K, Keller PM, Risch L, Risch M, Bodmer T. Comparison of the diagnostic performance of qPCR, Sanger sequencing, and whole-genome sequencing in determining clarithromycin and levofloxacin resistance in *Helicobacter pylori*. Front Cell Infect Microbiol 2020; 10: 596371.
13. Tshibangu-Kabamba E, Ngoma-Kisoko P de J, Tuan VP, Matsumoto T, Akada J, Kido Y, Tshimpi-Wola A, Tshiamala-Kashala P, Ahuka-Mundeke S, Ngoy DM, Disashi-Tumba G, Yamaoka Y. Next-generation sequencing of the whole bacterial genome for tracking molecular insight into the broad-spectrum antimicrobial resistance of *Helicobacter pylori* clinical isolates from the Democratic Republic of Congo. Microorganisms 2020; 8: 887.
14. Alzoubi H, Al-Mnayyis A, Al Rfoa I, Aqel A, Abu-Lubad M, Hamdan O, Jaber K. The Use of 13C-Urea Breath Test for Non-Invasive Diagnosis of *Helicobacter pylori* Infection in comparison to endoscopy and stool antigen test. Diagnostics (Basel) 2020; 10: 448.
15. Ojetti V, Saviano A, Petruzziello C, Brigida M, Pignataro G, Riccioni ME, Covino M, Candelli M, Saviano L, Barone F, Piccioni A, Franceschi F. 13C urea breath test to identify *Helicobacter pylori* Infection in patients with upper gastrointestinal bleeding admitted to the Emergency Department. Eur Rev Med Pharmacol Sci 2021; 25: 804-811.
16. Mizukami K, Katsuta M, Okamoto K, Fukuda K, Ogawa R, Kawahara Y, Hirashita Y, Sato Y, Fukuda M, Okimoto T, Kodama M, Fujioka T, Murakami K. Influence of acotiamide on 13C-urea breath test for *Helicobacter pylori* diagnosis. J Clin Biochem Nutr 2020; 67: 332-337.
17. Beresniak A, Malfertheiner P, Franceschi F, Liebaert F, Salhi H, Gisbert JP. *Helicobacter pylori* "Test-and-Treat" strategy with urea breath test: A cost-effective strategy for the management of dyspepsia and the prevention of ulcer and gastric cancer in Spain-Results of the Hp-Breath initiative. Helicobacter 2020; 25: e12693.
18. Fang YJ, Chen MJ, Chen CC, Lee JY, Yang TH, Yu CC, Chiu MC, Kuo CC, Weng YJ, Bair MJ, Wu MS, Luo JC, Liou JM. Accuracy of rapid *Helicobacter pylori* antigen tests for the surveillance of the updated prevalence of H. pylori in Taiwan. J Formos Med Assoc 2020; 119: 1626-1633.

19. Bénéjat L, Buissonnière A, Ducournau A, Mégraud F, Bessède E, Lehours P. Evaluation of RIDASCREEN® and RIDA®QUICK Helicobacter kits for *Helicobacter pylori* detection in stools. Eur J Clin Microbiol Infect Dis 2020; 39: 1941-1943.
20. Halland M, Haque R, Langhorst J, Boone JH, Petri WA. Clinical performance of the H. PYLORI QUIK CHEKTM and H. PYLORI CHEKTM assays, novel stool antigen tests for diagnosis of *Helicobacter pylori*. Eur J Clin Microbiol Infect Dis 2021; 40: 1023-1028.
21. Han Y, Dai W, Meng F, Gan X, Liu M, Deng X, Li Y, Wang G. Diagnosis of *Helicobacter pylori* infection in the elderly using an immunochromatographic assay-based stool antigen test. Microbiologyopen 2020; 9: e1102.
22. Leonardi M, La Marca G, Pajola B, Perandin F, Ligozzi M, Pomari E. Assessment of real-time PCR for *Helicobacter pylori* DNA detection in stool with co-infection of intestinal parasites: a comparative study of DNA extraction methods. BMC Microbiol 2020; 20: 131.
23. Butt J, Blot WJ, Shrubsole MJ, Varga MG, Hendrix LH, Crankshaw S, Waterboer T, Pawlita M, Epplein M. Performance of multiplex serology in discriminating active vs past *Helicobacter pylori* infection in a primarily African American population in the southeastern United States. Helicobacter 2020; 25: e12671.
24. Mäki M, Söderström D, Paloheimo L, Hendolin P, Suovaniemi O, SyrjÄnen K. *Helicobacter pylori* (Hp) IgG ELISA of the new-generation GastroPanel® Is highly accurate in diagnosis of Hp-Infection in gastroscopy referral patients. Anticancer Res 2020; 40: 6387-6398.
25. Takayama T, Suzuki H, Okada K, Yamada T, Maruo K, Saito Y, Mizokami Y. Prediction of true *Helicobacter pylori*-uninfected status using a combination of age, serum antibody and pepsinogen: Logistic regression analysis. PLoS One 2020; 15: e0240040.
26. Kusano C, Gotoda T, Ikehara H, Suzuki S, Shibuya H, Horii T, Arata S, Dohmen T. The accuracy of the serum antibody test for *Helicobacter pylori* Infection among junior high school students. Digestion 2021; 102: 155-160.
27. Akada J, Tshibangu-Kabamba E, Tuan VP, Kurogi S, Matsuo Y, Ansari S, Doohan D, Phuc BH, Subsomwong P, Waskito LA, Binh TT, Nguyen LT, Khien VV, Dung HDQ, Miftahussurur M, Syam AF, Tshering L, Vilaichone RK, Mahachai V, Ratanachu-Ek T, Shrestha PK, Yee TT, Htet K, Aftab H, Matsuhisa T, Uchida T, Okimoto T, Mizukami K, Kodama M, Murakami K, Takahashi N, Yamaoka Y. Serum *Helicobacter pylori* antibody reactivity in seven Asian countries using an automated latex aggregation turbidity assay. J Gastroenterol Hepatol 2021. doi: 10.1111/jgh.15467. Online ahead of print.
28. Bosch DE, Krumm N, Wener MH, Yeh MM, Truong CD, Reddi DM, Liu Y, Swanson PE, Schmidt RA, Bryan A. Serology Is more sensitive than urea breath test or stool antigen for the Initial diagnosis of *Helicobacter pylori* gastritis when compared with histopathology. Am J Clin Pathol 2020; 154: 255-265.
29. Hortelano I, Moreno Y, Moreno-Mesonero L, Ferrús MA. Deep-amplicon sequencing (DAS) analysis to determine the presence of pathogenic Helicobacter species in wastewater reused for irrigation. Environ Pollut 2020; 264: 114768.