1130-0108/2006/98/7/542-549
REVISTA ESPAÑOLA DE ENFERMEDADES DIGESTIVAS

REV ESP ENFERM DIG (Madrid)
Vol. 98. N.° 7, pp. 542-549, 2006

Is the string test a useful alternative to gastroscopy with biopsy for *H. pylori* identification?

A. J. del Pozo García and J. P. Gisbert[1]

Services of Digestive Diseases. Hospital Universitario de Guadalajara and [1]Hospital Universitario de La Princesa. Madrid. Spain

Del Pozo García AJ, Gisbert JP. Is the string test a useful alternative to gastroscopy with biopsy for H. pylori identification? Rev Esp Enferm Dig 2006; 98: 542-549.

INTRODUCTION

The diagnosis of *Helicobacter pylori (H. pylori)* infection may be reached using a number of both "direct" and "indirect" methods (1). The former rely on the "direct" identification of this organism via the histologic and microbiologic study of samples obtained with gastric biopsy. Therefore, they require endoscopy, which is certainly inconvenient for patients. This inconvenience and the relatively high cost of endoscopy has prompted the search for simpler alternatives, particularly in developing countries (2). "Indirect" methods are based upon the study and detection of selected bacterial characteristics, including the organism's ability to hydrolyze urea –property underlying the ^{13}C-urea breath test (1)– and the host's immune system response to infection (detection of specific antibodies using serologic tests). As a result, these "non-invasive" techniques add to their diagnostic accuracy the benefits of lower aggression and better tolerability by patients, but also the drawback that virulence or strain resistance patterns cannot be studied.

Thus far, when aiming at *H. pylori* collection for the performance of more or less complex techniques such as microscopy, culture, DNA analysis, cytotoxin screening, strain differentiation, or antimicrobial susceptibility analyses, endoscopy with gastric biopsy sampling was mandatory. The alarming, progressive increase of bacterial resistance rates has led to an increasing number of failed eradication procedures –approximately 20% of therapy-*naïve* individuals (3). Such lost efficacy makes sometimes advisable the collection of samples for culture, so that an antibiogram may be performed, and a more effective therapy is initiated according to bacterial susceptibility. Regarding this, there is some consensus on the performance of *H. pylori* cultures following a second failed eradication (4), while some authors have recommended this even prior to the index therapy, based on this strategy's higher cost-benefit ratio (5).

As resistance rates keep increasing, there is a growing need for a pre-treatment routine susceptibility test –as little aggressive as possible and efficient enough for *H. pylori* detection. The enterotest or string test is a method including the ingestion of a capsule attached to a long string, which is then extracted and analyzed for gastric secretions. This review attempts to assess the role of the string test in the diagnosis of *H. pylori* infection.

DESCRIBING THE TECHNIQUE

The enterotest or string test is a technique that was designed decades ago to collect fluids from the upper small bowel through the stomach, and was thus particularly used in the pediatric setting to obtain trophozoites in the study of giardiasis and other parasitoses. In 1995, some authors (6,7) started using this test for *H. pylori* cultures. In this test, a fasting patient ingests a small (7 mm) gelatin capsule attached to a 90-140 cm long nylon string with a highly absorbent 60 cm long distal segment; the string is placed within the capsule, and unwinds during ingestion. Upon reaching the stomach, the gelatin capsule dissolves and the string absorbs gastric secretions (approximately 0.5 ml per 10 cm) (8).

Recibido: 18-01-06.
Aceptado: 07-02-06.

Correspondencia: Javier P. Gisbert. Playa de Mojácar, 29. Urb. Bonanza. 28669 Boadilla del Monte. Madrid. Fax: 913 093 911. e-mail: gisbert@meditex.es

After a 30-180 minutes' interval, during which the patient may only take water and must remain in relative rest, the gastric juice-impregnated string is pulled out the mouth. This extraction must avoid contact between the string and the tongue and teeth; the string must then be pulled in a sustained, gentle manner, rather than in steps, with the patient keeping his or her mouth open during the process. To avoid sample contamination, mouthwahes using antiseptic solutions are recommended before extraction, as well as a careful, sterile manipulation of the string (8,9). In a recent study by Marshall et al., patients using chlorhexidine washes before extraction improved culture efficacy rates for *H. pylori* from 39 to 75% (9). Once out of the body, the string is proximally cut and the initial 30-cm segment is discarded, as it is non-absorbent and usually appears contaminated by nasopharyngeal flora.

No definite protocol has been developed yet for sample processing, but it is long known that *H. pylori* may survive below 10 ºC beyond 48 h prior to culture, and that greater success rates are reported for such cultures when air exposure is minimized during sample processing, handling, and transportation (10). The procedure described by Samuels et al. may be used, in view of its thoroughness and good results (11). Using this method, the remaining string is submerged in a sterile tube with 5-10 ml of isotonic saline for one minute, which reduces bacterial colonization at the expense of a decrease in the number of *H. pylori* colonies. The sample is stored at -70 ºC until processing, when it will be defrosted in 10 ml of saline, plus centrifugation at 3,000 g for 10 min. The supernatant is discarded, and the capsule is again suspended in 0.5 ml of saline, and employed for culture and/or polymerase chain reaction (PCR).

The string test is usually well tolerated, but mild oropharyngeal discomfort (cough, sore throat) may occur during or after the procedure, and nausea is not uncommon during extraction. In one study, 73% of patients reported that an enterotest was preferable to gastroscopy (12).

ENTEROTEST RELIABILITY IN THE DIAGNOSIS OF *H. PYLORI* INFECTION

According to studies published so far, the sensitivity of the enterotest for the detection of *H. pylori* oscillates between 37 and 97%, but varies a lot (widely) depending on the performance and processing technique (6,8,9,11-20) (Table I), as well as on the different string tests commercially available. Culture specificity from samples obtained using the enterotest reaches 100%. The low sensitivity reported by some authors has been attributed to various variables such as: a) bacterial overgrowth from the flora; b) failed collection of enough organism colonies using the string; or c) organism death during handling and processing (19). In order to attempt and prevent bacterial overgrowth from the oral and nasal pharyngeal flora, *H. pylori*-specific culture media (9,11) must be used, in addition to the above-mentioned extraction technique.

ENTEROTEST AND CULTURE

The first study to compare the effectiveness of *H. pylori* cultures between the enterotest and antral biopsy was reported by Pérez-Trallero et al. in 1995 (6). Both tech-

Table I. Diagnostic efficacy of the string test in studies reported so far

Author	*Year*	*No. of patients*	*Technique*	*Sensitivity (%)*	*Specificity (%)*	*PPV (%)*	*NPV (%)*
Domínguez et al. (15)	2000	29	PCR	79	100	100	75
Ferguson et al. (14)	1999	22	Culture	59	92	83	77
			PCR	63	100	100	80
Kopanski et al. (12)	1996	128	Culture	66	100	100	40
Leodolter et al. (22)	2005	42	Culture	81	100	100	88
Leong et al. (18)	2003	30	Culture	38	100	100	41
Parejo et al. (13)	1998	27	Culture	37	100	100	57
		27	PCR	93	100	100	92
Pérez-Trallero et al. (6)	1995	43	Culture	58	100	100	70
Roth et al. (17)	2001	40	PCR	70	100	100	37
Samuels et al. (11)	2000	33	Culture	97	100	100	86
Torres et al. (16)	2001	16	Culture	75	100	100	75
Wang et al. (19)	2003	34	PCR	94	96	92	86
Windsor et al. (9)	2005	45	Culture	75	100	100	0
Yoshida et al. (8)	1998	114	PCR	76	97	97	71
Total (*)		386	Culture	65	99	98	59
Total (*)		266	PCR	79	99	98	73
Total (*)		652	Culture and PCR	70	99	98	65

PPV: positive predictive value; NPV: negative predictive value; PCR: polymerase chain reaction; (*): weighted mean value.

niques were simultaneously compared in 36 adult patients with clinically suspected peptic ulcer or chronic gastritis. Samples obtained using both techniques were cultured on *Brucella* agar supplemented by 7% equine hemolyzed blood, and 1% Vitox (Oxoid) specific for vancomycin (15 μg/mL) and trimethoprim (5 μg/mL), as well as in this same medium in the absence of antibiotics. Plates were incubated at 37 ºC under microaerophilic conditions with 5% oxygen, 7% carbon dioxide, 2% hydrogen, and 86% nitrogen, and 80% humidity for 7 days. Cultures were deemed positive if colonies developed with a typical *H. pylori* morphology and positive oxidase, catalase, and urease activity following staining with acridine orange. Results showed a higher sensitivity in the group where gastric samples were obtained by biopsy, and consistency between both tests was moderate (21). Enterotest cultures showed a greater growth of flora other than *H. pylori* when compared to biopsy. The addition of 10 μg/mL of nalidixic acid to selective media increased sensitivity in the enterotest group. Since this report, the diagnostic efficacy of enterotest has been assessed in various *H. pylori*-selective culture media, and results have been thus inconsistent. Upon a review of the literature, we estimated that the mean sensitivity of enterotest-related samples is 65% (Table I), which is rather poor when compared to that of standard diagnostic methods. However, the study by Amy Samuels et al. (11) obtained a high sensitivity –97%– as a result of careful methodology and the use of three selective culture media: Wilkins-Chalgren agar with Dent (Oxoid) supplementation, colistin-nalidixic agar with Dent supplementation, and Skirrow agar.

ENTEROTEST AND PCR

The previous demonstration that the use of PCR for gastric biopsy –based upon restriction fragment length polymorphism (RFLP)– may serve to characterize and identify *H. pylori* strains infecting the stomach (22) has led to the application of this technique on enterotest-collected samples. The use of PCR in the string test considerably improves sensitivity by the sequencing of *H. pylori*-specific DNA (12,14-16,18,23), and has increased it in a recent study from 37% when based on cultures exclusively to 93% when PCR was added (13). Thus, following our review of the literature we estimated that the mean sensitivity of PCR on enterotest-collected samples for the identification of *H. pylori* is 79%, and increases by 14% culture sensitivity. As an added benefit, PCR allows to tell recrudescence apart from true reinfection following *H. pylori* eradication by comparing extant strains before and after therapy (23,24).

The major drawback of PCR is that it cannot be performed in all hospitals, since this technique requires sophisticated equipments and experienced personnel; furthermore, its use greatly increases costs. On the other hand, PCR –in contrast with culture– does not allow the performance of microbial susceptibility studies (19).

On the contrary, the fact that PCR may characterize the various *H. pylori* strains may help measure the differing virulences of these strains, and evaluate the potential mechanisms of infection transmission.

ENTEROTEST INDICATIONS

The availability of an "indirect" test for *H. pylori* diagnosis such as the ^{13}C urea breath test, which is fast, almost devoid of adverse effects, well tolerated, and relatively affordable (25,26), would restrict the string test to just a few indications. Thus, in yet undiagnosed patients, it seems advisable that a breath test be previously performed to confirm the presence of *H. pylori*, and that the string test be reserved for cases with confirmed infection (12). However, under what circumstances would this be useful? The string test may play a role in a number of clinical situations: a) after a failed eradication therapy course with culture and antibiogram purposes, so that appropriate antibiotics may be rationally selected and gastroscopy is spared (7); b) when a PCR is needed whatever the cause, without recourse to gastroscopy; and c) in population-based epidemiologic studies, to determine the prevalence of infection in healthy individuals for populations lacking breath-test technology in a minimally invasive manner, even if serology is a simpler option in such cases.

As previously seen, there is some consensus in indicating a culture and antibiogram after two failed *H. pylori* eradication treatments (4). Thus far, under such circumstances, patients underwent a new gastroscopy and gastric biopsy for sample culturing. The string test may be an alternative to gastroscopy in view of its scarce aggressiveness and better tolerability, especially in patients unwilling to undergo endoscopy or with a high risk for complications arising from their previous situation: altered anatomy (Zenker's diverticulum) or associated comorbidity (anticoagulating therapy, hemorrhagic diathesis, severe cardiorespiratory disturbances, immunodeficiency). A recent study (22) compared the efficacy of *H. pylori* cultures and the analysis of bacterial susceptibility to clarithromycin and metronidazole in patients with failed eradication –documented with a breath test– between samples obtained with the string test and samples collected by endoscopic biopsy, and found little differences in favor of biopsy; the authors concluded that using a string test under such circumstances may reduce the number of endoscopies by up to 80%, with considerable economic savings.

The role of the string test in directly documenting eradication success remains to be assessed, but breath testing has obvious advantages –lack of adverse effects, easy to perform, acceptable costs after initial investment (mass spectrometer). Anyway, should the sensitivity of the string test be optimized in the absence of PCR, the

role of this test would surely have greater preponderance.

The "test and treat" strategy (diagnosing and treating *H. pylori* infection) relies on the early use of an indirect test (serology or breath test) (27). There are increasingly more arguments –based both on decision analysis models and prospective studies– supporting the use of such a strategy, as it is associated with a reduced number of endoscopies and considerable savings (27). Hence, the II Spanish Consensus Conference on *H. pylori* infection (28) has recently supported the "test and treat" strategy as a valid alternative for younger patients with dyspepsia and no "alarm" signs. The role of the string test as an early component of this strategy may be approached. Some authors (29,30) advocate for a fast urease test (CLOtest) on enterotest-collected samples rather than a previous breath test for the study of only positive samples, which would reduce costs. However, the latter alternative has the limitation of potential false positive results due to the presence of contaminating flora from the upper respiratory tract (30,31).

CONCLUSIONS

To summarize, the string test is a minimally invasive technique that has been used for *H. pylori* identification during the past few years, and that may play a role in the diagnosis of this infection because of its user-friendliness (it may be used in both pediatric and elderly patients), harmlessness, good acceptance by patients, and reduced costs (provided no PCR is associated). However, thus far, and pending definitive validation, its use should be restricted to research studies. A potential indication may be antimicrobial-oriented culturing from enterotest-collected samples in patients with breath test-documented eradication failure, and in cases where gastroscopy is better avoided. Finally, the recently reported higher sensitivity rates in enterotest-related cultures, together with an increasingly simplified processing of samples and reduced PCR costs, may extend the future role of the string test in the diagnosis of *H. pylori* infection.

ACKNOWLEDGEMENTS

This review was partly supported by a grant from Instituto de Salud Carlos III (C03/02).

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