JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2009, p. 3980–3984 Vol. 47, No. 12
0095-1137/09/$12.00 doi:10.1128/JCM.01204-09

Improved Performance of a Rapid Office-Based Stool Test for Detection of *Helicobacter pylori* in Children before and after Therapy▽

C. Prell,[1] S. Osterrieder,[1] C. Lottspeich,[2] A. Schwarzer,[1] H. Rüssmann,[3] G. Ossiander,[1] and S. Koletzko[1]*

Dr. von Haunersches Kinderspital, Ludwig-Maximilians-University Munich, Lindwurmstraße 4, 80337 Munich, Germany[1]; Max von Pettenkofer Institute for Hygiene and Medical Microbiology, Ludwig-Maximilians-University Munich, Pettenkoferstraße 9a, 80336 Munich, Germany[2]; and HELIOS Klinikum Emil von Behring, Institute for Microbiology, Immunology and Laboratory Medicine, Walterhöferstraße 11, 14165 Berlin, Germany[3]

Received 19 June 2009/Returned for modification 20 August 2009/Accepted 9 October 2009

A modified version of a rapid office based one-step monoclonal immunoassay for detection of *Helicobacter pylori* antigen in stool samples from children was evaluated against biopsy specimen-based methods and compared to a monoclonal enzyme immunoassay using the same antigen. Blinded stool samples from 185 children (0.3 to 18.2 years) were investigated at the time of upper endoscopy prior to anti-*H. pylori* therapy; 62 children were *H. pylori* infected and 123 noninfected according to predefined reference standards. Samples obtained 6 to 8 weeks after anti-*H. pylori* therapy were available from 58 children (3.8 to 17.7 years) and were compared to results of the [^{13}C]urea breath test (14/58 were positive). The rapid stool tests were performed by two independent readers. Of 243 rapid tests performed, 1 (0.4%) was invalid for technical reasons. Equivocal results (very weak line) were reported 16 times by reader 1 and 27 times by reader 2. When equivocal results were considered positive, the two observers agreed on 76 positive and 160 negative results and disagreed on 7 samples (2.9%). The sensitivity was 90.8% for reader 1 and 85.5% for reader 2, and the specificity was 91.0% and 93.4%, respectively. The monoclonal enzyme immunoassay revealed a sensitivity and specificity of 94.7% and 97.6%, respectively. The modified chromatographic immunoassay is a good alternative in settings or situations when the monoclonal enzyme immunoassay or the [^{13}C]urea breath test are not available or feasible. In order to improve sensitivity, very weak lines should be considered positive test results.

Several noninvasive methods are available for the diagnosis of *H. pylori* infection (5, 14). Serological tests are not appropriate, since they cannot distinguish between a present and previous infection and, in addition, they have a low sensitivity in children younger than 12 years of age (6, 13). The [^{13}C]urea breath test (UBT) is the preferred noninvasive diagnostic tool and gives excellent performance for both adults and children, but specificity decreases in very young and mentally disabled children who are not able to cooperate with the test procedure (10, 11, 25). So far, tests for detection of *H. pylori* antigen in stool samples are the only noninvasive diagnostic tools which do not show an age dependence for the diagnostic accuracy (14, 15). This makes stool tests very attractive, particularly for young children and for epidemiological studies. Several tests have been developed, but validation studies showed differences in performance. An enzyme immunoassay (EIA) based on polyclonal antibodies that was developed by the Meridian Company has been validated in several studies, with controversial results (17, 20, 24). Lack of accuracy is obviously related to intertest variability (19). In contrast, EIA based on monoclonal antibodies showed consistently excellent results, with very high sensitivity and specificity in both children and adults (15, 21). A meta-analysis with head-to-head comparison has judged the monoclonal EIA superior to the polyclonal EIA (8).

Recently, we reported on the performance of a one-step monoclonal chromatographic immunoassay for detection of *H. pylori* antigen in stool samples from symptomatic children compared to the results of a well-established monoclonal EIA using the same antigen, namely, the catalase of *H. pylori* (22). Evaluation against biopsy specimen-based diagnostic methods showed a moderate sensitivity but a good specificity. After publication of the data, the manufacturer modified the tests. The aim of this study was to evaluate this new version of the rapid office-based one-step stool test in symptomatic children against invasive diagnostic methods and to compare the results with those of the monoclonal EIA.

MATERIALS AND METHODS

Patients. For the evaluation before treatment, stool samples from 185 symptomatic children (mean age, 10.2; range, 0.3 to 18.2 years) were frozen at −20°C at the time of endoscopies. All 185 patients had never been treated for *H. pylori* infection in the past. For 58 *H. pylori*-infected patients (mean age, 10.0; range, 3.8 to 17.7 years), stool samples were obtained 6 to 8 weeks after anti-*H. pylori* therapy and within 2 days of performing a UBT to monitor the success of therapy. Children were excluded if they took antibiotics or acid-suppressive drugs (proton pump inhibitor, H_2 receptor antagonists, antacids, or bismuth preparation) within 4 weeks prior to testing or if the *H. pylori* status was not clearly defined as described below.

The study protocol was approved by the ethical committee of the Ludwig-Maximilians-University, Munich, Germany.

Definition of *H. pylori* status. Two biopsy specimens each were taken during upper endoscopy from the gastric antrum and corpus for histological examina-

* Corresponding author. Mailing address: Dr. von Haunersches Kinderspital, Ludwig-Maximilians-University Munich, Lindwurmstraße 4, 80337 Munich, Germany. Phone: 49 89 5160 7854. Fax: 49 89 5160 7898. E-mail: sibylle.koletzko@med.uni-muenchen.de.

▽ Published ahead of print on 21 October 2009.

TABLE 1. Proportion of children in different age groups pre- or posttreatment

Age (yr)	% of children with indicated infection status (no. with status/total no.)			
	Pretreatment		Posttreatment	
	Positive	Negative	Positive	Negative
≤6	16.7 (8/48)	83.3 (40/48)	25.0 (2/8)	75.0 (6/8)
6–12	40.0 (28/70)	60.0 (42/70)	22.9 (8/35)	77.1 (27/35)
≥12	38.8 (26/67)	61.2 (41/67)	26.7 (4/15)	73.3 (11/15)
All	33.5 (62/185)	66.5 (123/185)	24.1 (14/58)	75.9 (44/58)

TABLE 2. Interobserver agreement between the two independent readers of the *RAPID* Hp StAR, with equivocal results counted separately

Result by reader 1	No. of samples with indicated result by reader 2[a]				Total no.
	−	+/−	+	++	
−	160	0	0	0	160
+/−	7	9	0	0	16
+	0	18	24	0	42
++	0	0	20	5	25
Total no.	167	27	44	5	243

[a] −, negative; +/−, equivocal; +, positive; ++, strongly positive.

tion. The specimens were formalin fixed and stained with hematoxylin and eosin and modified Giemsa. Local pathologists who were blinded for the results of the other tests viewed the specimens for the presence of *H. pylori*. One antral specimen was obtained for the rapid urease test and another for bacterial culture; this specimen was transported to the local microbiological laboratory in transport medium and processed within 4 hours. UBT was performed according to a standardized protocol, described previously (11). The test was considered positive when the delta over baseline value was ≥5‰.

In the pretreatment group, the *H. pylori* status was defined as positive when culture and at least two other tests (histology, rapid urease test, and UBT) were positive. The *H. pylori* status was considered to be negative if all tests performed gave negative results.

For ethical reasons and in accordance with the consensus statement of the pediatric task force group on *H. pylori* infection (7), the success of therapy was assessed by a UBT after treatment. The breath test was performed 6 to 8 weeks after the end of therapy. The results were compared with the results of the stool test and EIA.

Stool antigen tests. Parents were asked to bring a stool sample from their child at the time of endoscopy, before any therapy was initiated. Treated children had another stool sample delivered 6 to 8 weeks after the end of eradication therapy, at the time of the second UBT. Stool samples were stored at −20°C until analysis. Both the EIA and the rapid stool test were performed on coded samples. All tests were performed according to the manufacturer's instructions. If a stool specimen was insufficient, the patient was excluded.

The improved rapid test (*RAPID* Hp StAR; Oxoid, Ltd., Hampshire, United Kingdom) is an immunochromatographic membrane-based assay using amplification technology for the determination of *H. pylori* antigen in stool samples. This test utilizes two different monoclonal anti-*H. pylori* antibodies. Using the applicator stick, a pea-sized sample (approximately 0.1 g) of thoroughly mixed stool was transferred into the predispensed sample diluent vials and homogenized for 15 s on a vortex mixer. Three hundred fifty microliters of the stool suspension was added to the test strip vial using the supplied Pasteur pipette. Care was taken to keep the sides of the vial clean. The test strip was immersed in the sample and was left to stand vertically at room temperature for 15 min. The appearance of one purple-pink line (control line) indicated the correct performance of the test. According to the manufacturer's guidelines, stool samples can be stored at 2 to 8°C for up to 5 days or indefinitely at −20°C before the test.

Results were read visually within 5 min after the end of the incubation period. Results were judged as follows. A negative test result was only one pink band (control line). A positive test result was a distinguishable pink band (test line) in addition to the control line. The appearance of any pink test line was considered a positive result. Very weak, hardly visible test lines were considered equivocal. An invalid test result was the absence of the control line, with or without a visually detectable test line.

The results of the rapid stool test were read by two independent observers (reader 1 and reader 2) who classified the results of the coded samples as negative, positive or strongly positive, equivocal, or invalid. All stool tests were performed without knowledge of the other test results.

For the EIA (Amplified IDEIA Hp StAR; Oxoid, Ltd., Hampshire, United Kingdom), 50 μl of supernatant of the diluted stool sample (0.1 g stool in 0.5 ml sample diluent) and, thereafter, 50 μl of conjugated monoclonal antibody solution were added to wells and incubated for 1 h at room temperature on a shaker. Unbound material was removed by washing five times with a washing buffer. After the washing, 100 μl of a substrate solution was added and incubated for 10 min. After the addition of 100 μl of a stopping solution, the results were read by spectrophotometry (450/630-nm double wavelength). According to the manufacturer's guidelines, an optical density (OD) of ≤0.150 was defined as a negative and an OD of >0.150 as a positive test result.

Statistical analysis. Sensitivity and specificity with confidence intervals, accuracy, and the likelihood ratio for a positive and negative stool test result were calculated against the defined *H. pylori* status as the reference standard (20). Correlations between age and OD values were analyzed by the Spearman rho test. Statistical analysis was performed using SPSS (Statistical Package for Social Sciences version 9.1; SPSS, Inc., Chicago, IL).

RESULTS

***H. pylori* status.** According to the predefined criteria, 62 (34%) of the 185 patients in the pretreatment group were *H. pylori* infected. In all of them, the infection was proven by a positive culture for *H. pylori*, a positive histology, and rapid urease test. The *H. pylori* status was negative in 123 (66%) children, with concordant negative results in all tests.

Of 58 patients tested 6 to 8 weeks posttreatment, 14 (24%) showed a positive UBT result and were considered treatment failures, while the remaining 44 children (76%) had clearly negative UBT results. The number of children in each age group in relation to their *H. pylori* status is given in Table 1.

Rapid stool test. One test was invalid (0.4%) due to technical reasons; the sample was retested and showed a negative result. The two independent observers agreed on 29 positive or strongly positive and 160 negative results. In nine cases, both judged the reading to be equivocal (Table 2). Equivocal readings were reported 16 times by reader 1 and 27 times by reader 2. On 45 occasions, the reading was controversial between the two investigators, but 20 tests showed only grading differences within positive test results (positive or strongly positive). Accordingly, 25 tests (10.2%) were not concordant between the two readers.

In a second approach, all equivocal results (very weak, hardly visible test lines) were considered to be positive. The two observers now agreed on 56 positive and 160 negative results (Table 3). On 27 occasions, the results varied, but again, 20 cases showed only different positive results (positive or strongly positive). When equivocal results were considered to be positive, only seven tests (2.9%) had discordant results (reader 1, positive; reader 2, negative).

Table 4 gives the corresponding OD values measured in the EIA of all samples which gave discrepant readings (assuming all equivocal results to be positive test results). Surprisingly, in all but four cases, the OD values were clearly above or below the cutoff of 0.150. On 19 occasions (7.8%), both readers

TABLE 3. Interobserver agreement between the two independent readers of the *RAPID* Hp StAR, with equivocal results counted as positive

Result by reader 1	No. of samples with indicated result by reader 2[a]			Total no.
	−	+	++	
−	160	0	0	160
+	7	51	0	58
++	0	20	5	25
Total	167	71	5	243

[a] −, negative; +, positive; ++, strongly positive.

misclassified the *H. pylori* status, whereas in 7 cases (2.9%), only one investigator had a reading that was discrepant from the infection status. Discordant results occurred in both the pre- and posttreatment group (12% and 10%, respectively). Age, gender, or *H. pylori* status had no relation to the accuracy of the results.

The results for sensitivity, specificity, and accuracy and likelihood ratios for the pre- and posttreatment groups against the predefined gold standard are given in Table 5.

EIA stool test. Eight of 243 tests (3.3%) were misclassified by the EIA. Three false-negative and four false-positive results occurred in the pretreatment group, and one false positive after therapy (Table 4). In four patients, the *H. pylori* status was misclassified in both the rapid test and EIA. The OD values clearly differentiated between *H. pylori*-infected (median, 3.180; all but seven patients had values of >0.500) and noninfected children (median, 0.021; all but six had values of <0.080). No significant correlation was seen between the age of the patient and the OD value in the *H. pylori*-positive ($r = -0.124$, $P = 0.280$) or negative ($r = 0.003$, $P = 0.966$) groups. Results for sensitivity and specificity (with 95% confidence intervals), accuracy, and likelihood ratios of the EIA are presented in Table 5.

TABLE 4. Discrepancies between readers in rapid stool test readings and between results for both rapid stool test and EIA versus the reference standard[a]

Treatment group	*H. pylori* status	EIA	OD	Result for sample by:	
				Reader 1	Reader 2
Pre	−	+[b]	0.660	−	−
Pre	−	−	0.019	+[b]	+[b]
Pre	−	−	0.020	++[b]	++[b]
Pre	−	−	0.027	+[b]	−
Pre	−	−	0.019	+[b]	+[b]
Pre	−	−	0.020	+[b]	+[b]
Pre	−	+[b]	0.329	−	−
Pre	−	−	0.031	+[b]	+[b]
Pre	−	−	0.027	+[b]	+[b]
Pre	−	−	0.020	+[b]	+[b]
Pre	−	−	0.037	+[b]	+[b]
Pre	−	−	0.028	+[b]	+[b]
Pre	−	−	0.038	+[b]	+[b]
Pre	−	−	0.019	+[b]	−
Pre	−	−	0.021	+[b]	+[b]
Pre	−	+[b]	0.167	−	−
Pre	−	+[b]	3.521	++[b]	++[b]
Pre	+	−[b]	0.042	−[b]	−[b]
Pre	+	−[b]	0.100	−[b]	−[b]
Pre	+	+	0.590	+	−[b]
Pre	+	+	0.292	+	−[b]
Pre	+	+	0.266	−[b]	−[b]
Pre	+	−[b]	0.030	−[b]	−[b]
Post	−	−	0.039	+[b]	−
Post	−	+[b]	0.667	−	−
Post	+	+	0.244	−[b]	−[b]
Post	+	+	0.774	−[b]	−[b]
Post	+	+	0.879	+	−[b]
Post	+	+	0.533	+	−[b]

[a] OD values of monoclonal EIAs are given. Equivocal test results were considered to be positive. −, negative; +, positive; ++, strongly positive.
[b] Results were discordant from the reference *H. pylori* status.

DISCUSSION

To our knowledge, this is the first biopsy specimen-based study applying the modified rapid one-step immunochromatographic assay to establish the diagnosis of *H. pylori* infection in comparison to the established biopsy specimen-based reference standard pretreatment and to monitor the success of anti-*H. pylori* therapy against the UBT. The rapid stool test is based on the same monoclonal antibody as the monoclonal EIA which was used for comparison. Our results confirmed the excellent performance of the monoclonal EIA both pre- and posttreatment in pediatric patients (8, 9, 15). All published trials with children using the monoclonal EIA consistently showed values of ≥95% for sensitivity and specificity. Therefore, in children, this monoclonal EIA (Amplified IDEIA Hp STAR, formerly Femtolab *H. pylori*) seems to be as accurate as the UBT (14). In particular, in younger or mentally disabled children, the stool test has the advantage that it does not require any cooperation of the patient. The antigen is stable at room temperature for at least 3 to 5 days. Therefore, the stool sample can be sent in by normal surface mail. This saves another visit to the doctor's office, which is needed for the UBT.

A major disadvantage of the rapid test is the interobserver variability and equivocal results due to very weak test lines. In our study, two observers interpreted the reading independently. Forty-five times, the reading differed between the two investigators, but in 20 cases, both saw positive results (positive or strongly positive). In 25 cases (10.2%), the readers disagreed due to equivocal results. The visual interpretation of rapid stool tests has been reported to be a problem for both adult (4) and pediatric patients (1, 22). When we considered all equivocal readings to be positive results, discordant results occurred in only seven samples.

The preparation of the stool specimens and the test procedure were improved compared to the procedures for using the former test (22). Only one test was invalid for technical reasons, with no control line appearing. In our previous study, stool suspensions were stored at 4°C overnight before performance of the rapid test, which could be a reason for the worse performance. Therefore, we recommend that the stool samples be prepared and the rapid test performed immediately.

In the present study, the improved office-based monoclonal chromatographic test showed a better sensitivity pretreatment, with results of 93.5% and 90.3% for the two readers, compared to 85.7% and 71.4% posttreatment. So far, the sensitivity of the modified test is higher than reported values in previous studies applying the former version (2, 3, 18, 22). However, for the

TABLE 5. Performance of the monoclonal antigen stool tests according to pre- and posttreatment group[a]

Treatment group	Test	% Sensitivity (95% CI[b])		% Specificity (95% CI[b])		% Accuracy		Positive likelihood ratio		Negative likelihood ratio	
		Reader 1	Reader 2	Reader 1	Reader 2	Reader 1	Reader 2	Reader 1	Reader 2	Reader 1	Reader 2
Pretreatment (n = 185)	RAPID Hp StAR	93.5 (84.6–97.5)	90.3 (80.5–95.5)	89.4 (82.8–93.7)	91.9 (85.7–95.5)	90.8	91.4	8.8	11.1	0.07	0.11
	IDEIA Hp StAR	93.5 (84.6–97.5)		97.6 (93.1–99.2)		96.2		38.9		0.07	
Posttreatment (n = 58)	RAPID Hp StAR	85.7 (60.1–96.0)	71.4 (45.4–88.3)	97.7 (88.2–99.6)	100 (92.0–100)	94.8	93.1	37.3	∞	0.15	0.29
	IDEIA Hp StAR	100 (78.5–100)		97.7 (88.2–99.6)		98.3		43.5		0	
Pre- and posttreatment (n = 243)	RAPID Hp StAR	90.8 (82.2–95.5)	85.5 (75.9–91.7)	91.0 (85.7–94.5)	93.4 (88.6–96.3)	91.0	91.0	10.1	13.0	0.10	0.16
	IDEIA Hp StAR	94.7 (87.2–97.9)		97.6 (94.0–99.1)		96.7		39.6		0.05	

[a] All equivocal results were considered to be positive.
[b] CI, confidence interval.

posttreatment evaluation, only 14 patients with treatment failure could be studied, resulting in large confidence intervals (Table 5). Another explanation for the lower sensitivity after eradication therapy could be the reduced antigen load after failed therapy. Krausse et al. reported a lower sensitivity of the previous version of the rapid stool test for older patients compared to its sensitivity with patients below 45 years of age and speculated that the bacterial load in elderly people may decrease due to mucosal atrophy (18). In contrast to the rapid stool test, the monoclonal EIA was not negatively affected with respect to sensitivity in children with treatment failure. Our results are in agreement with a recent meta-analysis, including 22 studies covering 2,499 patients prior to and 957 patients following therapy, which showed comparable calculated pre- versus posttreatment sensitivities (percent sensitivity and 95% confidence interval, 0.94 [0.93 to 0.95] and 0.93 [0.89 to 0.96], respectively) (8).

A major advantage of fecal tests is the independence of the test accuracy from the child's age. While both serology (6, 12) and UBT (10, 11, 25) perform with lower accuracy in young children, this is not the case for stool tests. Our study included a larger proportion of very young patients than recent studies of fecal antigen tests (16, 23). This age range is important to study transmission and evaluate preventive measures for infection in high-risk populations, such as children from developing countries. The results of this study confirm our previous findings that the OD values in infected and noninfected children do not correlate with age (15, 22).

In summary, the modified rapid monoclonal immunochromatographic stool antigen test was modified, which led to an improved sensitivity and interobserver variability. Following the improvement, the monoclonal office-based test is now a good alternative to assess the *H. pylori* status of children in settings where the UBT or EIA is not available, not feasible, or too expensive. The EIA stool test, based on a monoclonal antibody, is highly sensitive and specific in children of all age groups, as described before. This test is an excellent alternative to the UBT for assessing the *H. pylori* status of children and for monitorings the success of anti-*H. pylori* therapy even in a low-prevalence setting.

ACKNOWLEDGMENT

The study was financially supported by Oxoid, Ltd., Hampshire, United Kingdom.

REFERENCES

1. **Antos, D., J. Crone, N. Konstantopoulos, and S. Koletzko.** 2005. Evaluation of a novel rapid one-step immunochromatographic assay for detection of monoclonal *Helicobacter pylori* antigen in stool samples from children. J. Clin. Microbiol. **43:**2598–2601.
2. **Blanco, S., M. Forne, A. Lacoma, C. Prat, M. A. Cuesta, I. Latorre, J. M. Viver, G. Fernandez, S. Molinos, and J. Dominguez.** 2008. Comparison of stool antigen immunoassay methods for detecting *Helicobacter pylori* infection before and after eradication treatment. Diagn. Microbiol. Infect. Dis. **61:**150–155.
3. **Cherian, S., D. P. Burgner, C. F. Carson, F. M. Sanfilippo, A. G. Cook, and D. A. Forbes.** 2008. Diagnosis of Helicobacter pylori infection in a high-prevalence pediatric population: a comparison of 2 fecal antigen testing methods and serology. J. Pediatr. Gastroenterol. Nutr. **47:**130–135.
4. **Chisholm, S. A., C. L. Watson, E. L. Teare, S. Saverymuttu, and R. J. Owen.** 2004. Non-invasive diagnosis of *Helicobacter pylori* infection in adult dyspeptic patients by stool antigen detection: does the rapid immunochromatography test provide a reliable alternative to conventional ELISA kits? J. Med. Microbiol. **53:**623–627.
5. **Cirak, M. Y., Y. Akyon, and F. Megraud.** 2007. Diagnosis of *Helicobacter pylori*. Helicobacter **12**(Suppl. 1)**:**4–9.
6. **de Oliveira, A. M., G. A. Rocha, D. M. Queiroz, E. N. Mendes, A. S. de Carvalho, T. C. Ferrari, and A. M. Nogueira.** 1999. Evaluation of enzyme-linked immunosorbent assay for the diagnosis of Helicobacter pylori infection in children from different age groups with and without duodenal ulcer. J. Pediatr. Gastroenterol. Nutr. **28:**157–161.
7. **Drumm, B., S. Koletzko, and G. Oderda.** 2000. Helicobacter pylori infection in children: a consensus statement. J. Pediatr. Gastroenterol. Nutr. **30:**207–213.
8. **Gisbert, J. P., F. de la Morena, and V. Abraira.** 2006. Accuracy of monoclonal stool antigen test for the diagnosis of *H. pylori* infection: a systematic review and meta-analysis. Am. J. Gastroenterol. **101:**1921–1930.
9. **Granstrom, M., P. Lehours, C. Bengtsson, and F. Megraud.** 2008. Diagnosis of Helicobacter pylori. Helicobacter **13**(Suppl. 1)**:**7–12.
10. **Imrie, C., M. Rowland, B. Bourke, and B. Drumm.** 2001. Limitations to carbon 13-labeled urea breath testing for Helicobacter pylori in infants. J. Pediatr. **139:**734–737.
11. **Kindermann, A., H. Demmelmair, B. Koletzko, S. Krauss-Etschmann, B. Wiebecke, and S. Koletzko.** 2000. Influence of age on 13C-urea breath test results in children. J. Pediatr. Gastroenterol. Nutr. **30:**85–91.
12. **Kindermann, A., N. Konstantopoulos, N. Lehn, H. Demmelmair, and S. Koletzko.** 2001. Evaluation of two commercial enzyme immunoassays, testing immunoglobulin G (IgG) and IgA responses, for diagnosis of *Helicobacter pylori* infection in children. J. Clin. Microbiol. **39:**3591–3596.
13. **Kindermann, A., C. Tasch, T. Sauerwald, H. Rüssmann, H. Weigand, H. Demmelmair, and S. Koletzko.** 2000. Major differences of commercial ELISA to detect *H. pylori* infection in children. J. Pediatr. Gastroenterol. Nutr. **31**(Suppl. 2)**:**S148.
14. **Koletzko, S.** 2005. Noninvasive diagnostic tests for *Helicobacter pylori* infection in children. Can. J. Gastroenterol. **19:**433–439.

15. **Koletzko, S., N. Konstantopoulos, D. Bosman, A. Feydt-Schmidt, A. van der Ende, N. Kalach, J. Raymond, and H. Russmann.** 2003. Evaluation of a novel monoclonal enzyme immunoassay for detection of *Helicobacter pylori* antigen in stool from children. Gut **52:**804–806.
16. **Konstantopoulos, N., S. Koletzko, A. Feydt-Schmidt, H. Rüssmann, D. K. Bosman, A. van der Ende, N. Kalach, and J. Raymond.** 2001. Detection of H. pylori antigen in stool with a novel monoclonal EIA (FemtoLab H. pylori Cnx). J. Pediatr. Gastroenterol. Nutr. **33:**108. (Abstract.)
17. **Konstantopoulos, N., H. Russmann, C. Tasch, T. Sauerwald, H. Demmelmair, I. Autenrieth, and S. Koletzko.** 2001. Evaluation of the Helicobacter pylori stool antigen test (HpSA) for detection of Helicobacter pylori infection in children. Am. J. Gastroenterol. **96:**677–683.
18. **Krausse, R., G. Muller, and M. Doniec.** 2008. Evaluation of a rapid new stool antigen test for diagnosis of *Helicobacter pylori* infection in adult patients. J. Clin. Microbiol. **46:**2062–2065.
19. **Makristathis, A., W. Barousch, E. Pasching, C. Binder, C. Kuderna, P. Apfalter, M. L. Rotter, and A. M. Hirschl.** 2000. Two enzyme immunoassays and PCR for detection of Helicobacter pylori in stool specimens from pediatric patients before and after eradication therapy. J. Clin. Microbiol. **38:**3710–3714.
20. **Megraud, F.** 2005. Comparison of non-invasive tests to detect Helicobacter pylori infection in children and adolescents: results of a multicenter European study. J. Pediatr. **146:**198–203.
21. **Nguyen, T. V., C. Bengtsson, G. K. Nguyen, and M. Granstrom.** 2008. Evaluation of a novel monoclonal-based antigen-in-stool enzyme immunoassay (Premier Platinum HpSA PLUS) for diagnosis of Helicobacter pylori infection in Vietnamese children. Helicobacter **13:**269–273.
22. **Schwarzer, A., C. Lottspeich, H. Russmann, G. Ossiander, and S. Koletzko.** 2007. Evaluation of a novel rapid one-step monoclonal chromatographic immunoassay for detection of Helicobacter pylori in stool from children. Eur. J. Clin. Microbiol. Infect. Dis. **26:**475–480.
23. **van Doorn, O. J., D. K. Bosman, B. W. van't Hoff, J. A. Taminiau, F. J. ten Kate, and A. van der Ende.** 2001. Helicobacter pylori stool antigen test: a reliable non-invasive test for the diagnosis of Helicobacter pylori infection in children. Eur. J. Gastroenterol. Hepatol. **13:**1061–1065.
24. **Yang, H. R., and J. K. Seo.** 2008. *Helicobacter pylori* stool antigen (HpSA) tests in children before and after eradication therapy: comparison of rapid immunochromatographic assay and HpSA ELISA. Dig. Dis. Sci. **53:**2053–2058.
25. **Yang, Y. J., B. S. Sheu, S. C. Lee, and J. J. Wu.** 2007. More economic 25 mg 13C-urea breath test can be effective in detecting primary *Helicobacter pylori* infection in children. J. Gastroenterol. Hepatol. **22:**335–339.