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Evaluation of the rapid RIDAQUICK Campylobacter® test in a general hospital

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ABSTRACT

The study objective was to evaluate the effectiveness of the new rapid immunochromatographic test RIDAQUICK Campylobacter® (r-biopharm AG, Darmstadt, Germany) for the qualitative detection of *Campylobacter* antigens in pathologic feces from primary and specialist care patients. Three hundred feces samples were studied from patients with diarrhea, 50.6% from adults and 49.4% from children, which were received by our microbiology laboratory for coproculture. *Campylobacter* culture results, with or without PCR data, served as reference values for the comparative evaluation of RIDAQUICK Campylobacter® findings. *Campylobacter* was detected in 12.3% of samples. The diagnostic accuracy values of the RidaQuick Campylobacter® *versus* culture were: sensitivity of 87%, specificity of 97%, and positive and negative predictive values of 77% and 98%, respectively. RIDAQUICK Campylobacter® is a rapid test for the diagnosis of enteritis due to *Campylobacter* and could be an option for the clinical diagnosis of one of the main causes of bacterial enteritis in resource-limited settings.

1. Introduction

The genus *Campylobacter* belongs to the family Campylobacteriaceae (Hindiyeh et al., 2000) and contains 18 species, including *Campylobacter jejuni*, the cause of around 90% of acute *Campylobacter* enteritis cases in humans (Hindiyeh et al., 2000; Kawatsu et al., 2008; Granato et al., 2010). This zoonotic disease is prevalent worldwide and transmitted *via* water and food (Friedman et al., 2000; Granato et al., 2010).

C. jejuni and *Campylobacter coli* colonize the digestive tract of numerous animals, especially birds, and most infections are produced by the consumption of their undercooked meat (Granato et al., 2010). The incubation period of the enteritis ranges from 1 to 7 days, and its signs and symptoms are non-specific. These infections are generally self-limiting (Skirrow and Titus, 2000) and only rarely give rise to extra-intestinal disease (e.g., bacteremia, reactive arthritis, or Guillain-Barré syndrome) (Hannu et al., 2002; Granato et al., 2010; Floch et al., 2012). Hence, antibiotic treatment is not usually indicated for diagnosed patients (Giltner et al., 2013). Nevertheless, detection of this etiologic agent is important for the differential diagnosis with other potential causes of diarrhea (Tissari and Rautelin, 2007) and because the infection may persist. Furthermore, specific antibiotic treatment may be indicated in immunodepressed patients and in children and the elderly, showing an appreciable rate of resistance to fluoroquinolones (Hindiyeh et al., 2000; Tolcin et al., 2000; Granato et al., 2008; Arsenault et al., 2011). For these reasons, rapid etiologic detection is vital in the aforementioned populations.

The diagnosis of enteritis due to *Campylobacter* was traditionally performed using culture, visualization by Gram staining (fuchsin), and conventional biochemical identification methods such as API-systems (bioMerieux Vitek, Inc, Hazelwood, MO, USA). *Campylobacter* is a curved Gram-negative rod that requires microaerophilic incubation for up to 48 h at 42 °C using selective media (Bessède et al., 2011a). More recent diagnostic techniques include the detection of antigens (enzyme-linked immunosorbent assay or immunochromatography) or DNA in feces and matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry of the colonies (Bessède et al., 2011b; Martiny et al., 2013). The antigen detection methods are of particular interest because they yield results within hours or minutes and are easy to apply, especially using the immunochromatographic approach (Floch et al., 2012). In addition, *Campylobacter* antigens can persist in the clinical sample in the absence of viable microorganisms, allowing primary infections to be detected by this method when the bacteria are not viable, which is a major potential clinical advantage. However, highly variable results have been obtained using commercialized kits (Table 1), and further knowledge is required on the functioning of these kits in clinical samples to establish their usefulness in the diagnosis of this infectious enteritis.

The objective of this study was to evaluate the behavior of the new rapid immunochromatographic test RIDAQUICK Campylobacter®

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Table 1
Results of tests for detection of *Campylobacter* antigen.

Study	Test	Results	No. of samples	
Tolcin et al., 2000	ProSpecT *Campylobacter*® (Alexon-Trend, Ramsey, MN, USA): EIA microplate assay.	Se = 96%; Sp = 99%		164; GS: culture
Endtz et al., 2000		Se = 80%; Sp = 100%		30; GS: culture
Hindiyeh et al., 2000		Se = 89%; Sp = 99%; PPV = 80%; NPV = 99%		631; GS: culture
Dediste et al., 2003		Se = 89.1%; Sp = 97.7%; PPV = 78.3%; NPV = 99%		1205; GS: culture
Tissari and Rautelin, 2007	RidaScreen *Campylobacter*®			
(R-biopharm AG, Darmstadt, Germany): EIA microplate assay	Se = 69%; Sp = 87%		1050; GS: culture	
Kawatsu et al., 2008	Campy in-house: immunochromatographic assay	Se = 84.8%; Sp = 100%		222; GS: culture
Bessède et al., 2011a	In-house PCR	Se = 80%; PPV = 85%		242; GS: Culture or PCR + EIA
	Multiplex PCR Seeplex Diarrhea ACE Detection®	Se = 89%; PPV = 85%		
	RidaScreen *Campylobacter*®			
(R-biopharm AG, Darmstadt, Germany): EIA microplate assay.	Se = 91%; PPV = 85%			
	Premier CAMPY® (Meridian Bioscience, Inc. Cincinnati, OH, USA): EIA microplate assay.	Se = 95%; PPV = 80%		
	ImmunoCard STAT! Campy® (Meridian Bioscience, Inc.): immunochromatographic assay.	Se = 90%; PPV = 70%		
Dey et al., 2012		Se = 86%; Sp = 100%; GS: culture	Se = 90%; Sp = 100%; GS: PCR	463
Floch et al., 2012		PPV = 80.6%		609; GS: culture
Couturier et al., 2013		Se = 80%; Sp = 98%		500; GS: culture
Granato et al., 2010		Se = 98.4%; Sp = 94.2%; PPV = 92.6%; NPV = 98.8%; GS: culture	Se = 98.5%; Sp = 98.2%; PPV = 97.8%; NPV = 98.8%; GS: culture + PCR (discordant)	300
Giltner et al., 2013	Premier CAMPY® (Meridian Bioscience, Inc.: EIA microplate assay.	Se = 100%; Sp = 98%; PPV = 91%; NPV = 100%	60; GS: culture	
		Se = 75%; Sp = 96.5%; PPV = 42.9%; NPV = 99%	119; GS: culture	

EIA = enzyme immunoassay; Se = Sensitivity; Sp = Specificity; PPV = Positive predictive value; NPV = Negative predictive value; GS = Gold standard.

(r-biopharm AG, Darmstadt, Germany) in the detection of *Campylobacter* antigen in feces samples from patients with diarrhea.

2. Materials and methods

Between March and May 2013, 300 consecutive samples of fresh feces samples were studied from patients diagnosed with diarrhea in primary (57.6%) and specialist (42.4%) care; 50.6% were from adults and 49.4% from children under 14 years of age (Table 2). The samples were received by the Microbiology Laboratory of University Hospital Virgen de las Nieves, Granada (Spain), for coproculture and were simultaneously studied with the RIDAQUICK Campylobacter® test to detect the presence of *Campylobacter* antigens. This hospital is a reference center in Andalusia (southern Spain) serving a population of around 440,000 individuals.

Samples were gathered in sterile containers with no transport media and delivered to our laboratory under refrigeration (4 °C), with a maximum delay of 2 hours before their processing. They were processed for coproculture immediately on their reception by culture in CampyBAP® medium with 10% blood (Becton Dickinson, BD, Franklin Lakes, NJ, USA) using a 30-μg cefoxitin disk (BD BBL®) and incubated for 48 h at 42 °C in microaerophilic atmosphere (Campygen®, Oxoid, Basingstoke, UK). Suspicious colonies were identified by means of oxidase cytochrome tests (Difco, Detroit, MI, USA), Gram staining, and mass spectrometry using the Biotyper® system (Bruker Daltonics, Coventry, UK) (He et al., 2010). The samples were also seeded in the usual culture media for enteropathogens for 48 h (XLD® agar [BD] at 37 °C for recovery of *Salmonella*, *Shigella*, and *Plesiomonas*, and CIN® agar [BD] at 30 °C for recovery of *Yersinia* and *Aeromonas*) or 24 h (Selenito® broth [Difco] for recovery of *Salmonella*, followed by a subculture in Hektoen® agar [BD] at 37 °C). Colonies suspected of being enteropathogenic were identified by means of the Biotyper® system (Bruker Daltonics) and the usual biochemical tests (MicroScan; Siemens Healthcare, Rockville, MD, USA). Colonies identified as *Salmonella* were subjected to the agglutination test to determine the serogroup (Difco).

The RIDAQUICK Campylobacter® was used according to the manufacturer's instructions, mixing well 50 mg or 50 μL of the feces in a tube containing 0.5 mL of reagent A and 0.5 mL of reagent B. The mixture was incubated at room temperature for 5 min, and 150 μL of the supernatant was then placed in the sampling port of the device. After a 15-min interval at room temperature, the reading was made by 2 researchers using simple visual observation, and the result was accepted for evaluation when their readings coincided. The result was considered positive when violet red lines could be seen in the control (C) and test (T) bands, negative when the control line alone appeared, and invalid when no line could be observed.

All samples tested were kept at −80 °C in aliquots. In samples with discrepant culture and immunochromatography results, the presence of *Campylobacter* DNA was also investigated using RIDAGENE

Table 2
Distribution of feces samples and presence of microorganisms by origin and age of the patients studied.

Origin	No. of feces samples				*Campylobacter* positive		Other enteropathogen	
	Total	Child	Adult	With enteropathogen	Child	Adult	Child	Adult
Primary care	171	112	59	37	21[a]	7	10 (9[a] *Salmonella*; 1 *Aeromonas*)	0
Specialist care	129	36	93	11	1	8	2 (1 *Salmonella*; 1 *Aeromonas*)	0
Total	300	148	152	48	22	15	12	0

[a] One case with simultaneous isolation of *Campylobacter* and *Salmonella*.

Bacterial Stool Panel PCR® (r-Biopharm AG, Darmstadt, Germany), a qualitative multiplex real-time PCR assay (Mx3005p system; Strategene, La Jolla, CA, USA) that targets 16s-rDNA of *Campylobacter* spp. with a fluorogenic target-specific hydrolysis probe. Before the extraction (Maxwell DNA purification kit, Valencia, CA, USA), stool samples were diluted 1:3 with water, intensely vortexed, and centrifuged at 3000 rpm for 30 sec.

The diagnostic accuracy of the RidaQuick Campylobacter® test was evaluated by calculating the sensitivity, specificity, and positive and negative predictive values, with exact binomial interval of Clopper-Pearson. *Campylobacter* was considered present when the microorganism was isolated or when its DNA was detected in samples with contradictory culture and immunochromatography findings.

3. Results

Out of the 300 samples of feces studied (Table 2), the highest percentage with enteropathogens (12.3%) came from children aged less than 14 years referred by primary care centers. *Campylobacter* was detected in 37 (12.3%) of all samples, and the culture was positive (gold-standard method) for *C. jejuni* in 31 (83.78%) of these. A different enteropathogen was detected in 12 (8.11%) of the samples from children.

The culture and immunochromatography results differed in 12 samples (4% of the total; Table 3), which were therefore studied by PCR; 6 of these samples were culture-negative and PCR-positive and 1 was culture positive (*C. jejuni*) and PCR-negative. Comparisons between the immunochromatography and the culture and/or PCR results are exhibited in Table 4.

The 2 researchers independently gave the same result in all samples, and no sample was unreadable. There was no case in which the test was positive when the enteropathogen isolated was other than *Campylobacter*. The diagnostic accuracy values of the RidaQuick Campylobacter® *versus* culture were: sensitivity of 87% (95% confidence interval [CI]: 0.70–0.96), specificity of 97% (95% CI: 0.94–0.97), and positive and negative predictive values of 77% (95% CI: 0.60–0.90) and 98% (95% CI: 0.96–0.99), respectively. The diagnostic accuracy values *versus* culture *plus* PCR were: sensitivity of 89% (95% CI: 0.75–0.97), specificity of 99% (95% CI: 0.97–1.00), and positive and negative predictive values of 94% (95% CI: 0.81–0.99) and 98% (95% CI: 0.96–1.00), respectively.

Table 3
PCR results for samples with discrepant antigen detection and culture findings.

Sample number	Immunochromatography	Culture	PCR	Interpretation
1	+	−	−	−
2	+	−	+	+
3	−	+	+	+
4	+	−	+	+
5	+	−	−	−
6	+	−	+	+
7	+	−	+	+
8	−	+	+	+
9	+	−	+	+
10	−	+	−	+
11	+	−	+	+
12	−	+	+	+

Interpretation: culture *plus* PCR.

4. Discussion

Campylobacter is one of the main agents responsible for enteritis in our region, explaining our interest in the availability at primary and emergency care levels of a rapid, simple, and reliable test for its detection in feces samples. This could deliver major clinical benefits for the management of high-risk patients (Hannu et al., 2002; Granato et al., 2008; Floch et al., 2012) and for the early detection of outbreaks (Calciati et al., 2012) by detecting this agent in a rapid manner and with fewer false negatives due to inadequate sample transport.

Most patients with intestinal disorders produced by *Campylobacter* are seen in primary care centers and do not usually require antibiotic treatment or hospitalization. However, besides the need for a rapid test to reduce the morbidity, protect high-risk patients, and detect outbreaks, its availability at primary care level could facilitate therapeutic decision making at hospital level in patients referred for specialist treatment due to their clinical symptoms, contributing to relieving the pressure on emergency departments.

This type of test must show adequate diagnostic profitability and be easy to apply, and limitations in the results interpretation must be established. In the present study, application of the RIDAQUICK Campylobacter®, using only the material supplied by the manufacturer, may have helped to disclose the possible cause of the diarrhea at around 20 min after reception of the feces sample. The test was easy to perform and interpret, requires no specific training, and is not very costly (6 €), allowing its use to be recommended in primary and emergency care settings.

Discordant results were obtained for 12 (4%) of the 300 samples (Table 3). Four of these (positive culture and negative immunochromatography) can be attributed to a lack of sensitivity of the test; the subsequent PCR test was positive in 3 of these 4 samples but negative in one, which may be because the amount of specific DNA was below the limit of detection of this multiplex PCR.

A major limitation of our study is that PCR was only applied in cases of discrepancy between culture and rapid testing and not in cases of false-negative culture results due to suboptimal transport conditions or cases of false-negative rapid test results attributable to the potentially low sensitivity of these tests.

Table 4
Results of antigen detection by immunochromatography with RIDAQUICK Campylobacter® *versus* culture with or without PCR.

	RIDAQUICK Campylobacter®		
	Positive (no.)	Negative (no.)	Total (no.)
Culture			
Positive (no.)	27	4	31
Negative (no.)	8	261	269
Culture with PCR			
Positive (no.)	33	4	37
Negative (no.)	2	261	263
Total	35	265	300

The limitation of PCRs, alongside their laborious nature and high cost, makes immunochromatography of clinical interest. Six of the discrepant samples showed negative culture results but positive PCR results, supporting the use of these rapid tests on the first contact of patients with the health system to reduce the false negatives due to bacteria viability losses. It has been reported that sample transport conditions require optimization in order to achieve more adequate results in bacterial culture (Wang et al., 1983). There were only 2 cases of false positives using immunochromatography (0.66%); however, their clinical significance is difficult to determine without knowledge of the antigen detected in the kit.

Only 12 studies published in the English language have evaluated kits for the detection of *Campylobacter* antigen in feces. The sensitivity of the 8 enzyme immunoassays and 6 immunochromatographic kits ranged between 69% and 100% and the specificity between 87% and 100% (Table 1). The present study obtained poor sensitivity but adequate specificity. Only one of the aforementioned studies (Kawatsu et al., 2008) described the antibody used in the kit, a monoclonal antibody against a *C. jejuni* cell surface protein of 15 kDa.

In addition, this rapid test provides no information on the resistance pattern of the causative *Campylobacter* isolate, and resistance testing is important in the microbiological diagnosis of campylobacteriosis at a time of increasing macrolide resistance in *Campylobacter* spp. (Coker et al., 2002; Wieczorek and Osek, 2013).

In conclusion, detection of the *Campylobacter* antigen in pathological feces using RIDAQUICK Campylobacter® is an easily applied and rapid procedure that yields acceptable results for the clinical diagnosis of patients.

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