

Evaluation of Four Commercial Rapid Immunochematographic Assays for Detection of Cryptosporidium Antigens in Stool Samples: a Blind Multicenter Trial

Patrice Agnamey, Claudine Sarfati, Claudine Pinel, Meja Rabodoniriina, Nathalie Kapel, Emmanuel Dutoit, Cécile Garnaud, Momar Diouf, Jean-François Garin, Anne Totet, F. Derouin and for the ANOFEL Cryptosporidium National Network

J. Clin. Microbiol. 2011, 49(4):1605. DOI:
10.1128/JCM.02074-10.

Published Ahead of Print 2 February 2011.

Updated information and services can be found at:
<http://jcm.asm.org/content/49/4/1605>

These include:

REFERENCES

This article cites 18 articles, 8 of which can be accessed free at:
<http://jcm.asm.org/content/49/4/1605#ref-list-1>

CONTENT ALERTS

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), [more»](#)

Information about commercial reprint orders: <http://journals.asm.org/site/misc/reprints.xhtml>
To subscribe to to another ASM Journal go to: <http://journals.asm.org/site/subscriptions/>

Evaluation of Four Commercial Rapid Immunochromatographic Assays for Detection of *Cryptosporidium* Antigens in Stool Samples: a Blind Multicenter Trial[∇]

Patrice Agnamey,¹ Claudine Sarfati,² Claudine Pinel,³ Meja Rabodoniriina,⁴ Nathalie Kapel,⁵ Emmanuel Dutoit,⁶ Cécile Garnaud,³ Momar Diouf,⁷ Jean-François Garin,² Anne Totet,¹ and F. Derouin^{2*} for the ANOFEL *Cryptosporidium* National Network

Laboratory of Parasitology-Myology, University Hospital and University of Picardy Jules Verne, Amiens, France¹; Laboratory of Parasitology-Myology, Saint-Louis Hospital, Assistance Publique-Hôpitaux de Paris, and Université Denis Diderot, Paris, France²; Laboratory of Parasitology-Myology, Institut de Biologie Pathologie (IBP), Centre Hospitalier Universitaire Albert Michallon, Grenoble, France³; Laboratory of Parasitology and Tropical diseases, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, and Université Claude-Bernard Lyon 1, Lyon, France⁴; Ecosystème intestinal, probiotiques, antibiotiques (EA 4065), Université Paris Descartes, Paris, France⁵; Laboratory of Parasitology-Myology, Hospital University, Lille, France⁶; and Unité de Biostatistique, Direction de la Recherche Clinique et de l'Innovation, University Hospital Amiens, France⁷

Received 13 October 2010/Returned for modification 2 November 2010/Accepted 18 January 2011

In a multicenter study, potassium dichromate-preserved stools from patients infected with *Cryptosporidium parvum* ($n = 20$), *C. hominis* ($n = 20$), and other *Cryptosporidium* species ($n = 10$) and 60 controls were examined using four immunochromatographic assays. Assay sensitivity ranged between 50.1% and 86.7% for *C. parvum* and *C. hominis* but was <35% for other species.

Cryptosporidiosis is a common protozoan diarrheal disease in humans. It is usually diagnosed by microscopic detection of *Cryptosporidium* oocysts in stool specimens (3). Antigen detection by immunoassays has become a well-established aid to microscopic examination for the diagnosis of cryptosporidiosis. Good sensitivities and specificities have been reported for some of these tests in several comparative studies (8–11, 13, 14, 16). However, considerable progress has been made in the molecular characterization of *Cryptosporidium* since the development of these tests, resulting in the identification of at least seven human-infecting species (3, 18). *Cryptosporidium parvum* and *C. hominis* remain the two most frequent species detected with various levels of prevalence in different countries (2, 6, 7, 12, 15, 17) but with a high (up to 90%) predominance of *C. hominis* in tropical and developing countries (1, 4, 5, 18). Species other than *C. parvum* and *C. hominis* have also emerged as causes of cryptosporidiosis in both immunocompromised and immunocompetent patients (2, 4, 5). In this context, the sensitivity of immunoassays for the detection of *C. hominis* and other human-infecting species has to be assessed.

Fifty stool samples containing *Cryptosporidium* oocysts were provided by the French ANOFEL *Cryptosporidium* National Network (2). The diagnosis was established by microscopy, and then stool samples were preserved in 2.5% potassium dichromate (1:1 dilution) and sent to a centralized laboratory in aliquots for storage (4°C) and genotyping. The durations of storage before this study was performed ranged between <12

months ($n = 40$), 12 to 24 months ($n = 5$), and 24 to 40 months ($n = 5$). The *Cryptosporidium* species determined by PCR sequencing at the 18S ribosomal DNA locus (2) consisted of *C. parvum* ($n = 20$), *C. hominis* ($n = 20$), *C. felis* ($n = 6$), *C. meleagridis* ($n = 2$), *C. canis* ($n = 1$), and an unidentified *Cryptosporidium* sp. ($n = 1$). Five samples containing *Cyclospora* oocysts and 5 containing *Cystoisospora* (syn. *Isospora*) oocysts were also collected from the network. For the study, coded aliquots of each stool sample were sent to the following parasitology laboratories: Amiens University Hospital (Lab. A), Grenoble University Hospital (Lab. B), and Paris Saint-Louis Hospital (Lab. C). Each study center also provided 20 potassium dichromate-fixed negative controls derived from their own routine activity in which the absence of *Cryptosporidium* was confirmed by microscopy (all laboratories) and PCR (Lab. A). In each study center, the following immunoassay diagnostic kits were tested: RIDAQuick *Cryptosporidium* (R-biopharm Diagnostic; Germany); ImmunoCard STAT! *Cryptosporidium*/*Giardia* rapid assay (Meridian Bioscience Inc.); Crypto-Strip (Coris BioConcept; (Belgium), and Remel-Xpect *Cryptosporidium* (Remel Inc.). Stool samples were processed according to the instructions of the manufacturers, taking into account the initial 1:1 dilution in potassium dichromate. In each study center, the tests were performed by the same experienced staff members to reduce the risk of handling and reading errors.

Detailed results are presented in Table 1. Each center performed at least 317 tests. Ten invalid results (4 with Remel-Xpect and 6 with ImmunoCard STAT!) were found that were due to the absence of the positive-control line within the recommended time period. Statistical analyses were performed on valid test results. No positivity was observed for stools containing *Cystoisospora* or *Cyclospora* oocysts or for 59/60 of the

* Corresponding author. Mailing address: Laboratory of Parasitology-Myology, Saint-Louis hospital, 1 avenue Claude Vellefaux 75475 Paris Cedex 10, France. Phone: 33 1 42 49 95 01. Fax: 33 1 42 49 48 03. E-mail: francis.derouin@sls.ap-hop-paris.fr.

[∇] Published ahead of print on 2 February 2011.

TABLE 1. *Cryptosporidium* antigen detection using rapid immunoassays and potassium dichromate-fixed stool specimens^a

Species	RIDA Quick						Remel X/pect						ImmunoCard/STAT!						Crypto-Strip					
	No. of positive specimens/ laboratory			Mean %			No. of positive specimens/ laboratory			Mean %			No. of positive specimens/ laboratory			Mean %			No. of positive specimens/ laboratory			Mean %		
	Lab. A	Lab. B	Lab. C	Lab. A	Lab. B	Lab. C	Lab. A	Lab. B	Lab. C	Lab. A	Lab. B	Lab. C	Lab. A	Lab. B	Lab. C	Lab. A	Lab. B	Lab. C	Lab. A	Lab. B	Lab. C	Lab. A	Lab. B	Lab. C
<i>C. parvum</i> (n = 20)	15	15	14	73.3	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14
<i>C. hominis</i> (n = 20)	14	16	14	73.3	17	15	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
Other <i>Cryptosporidium</i> species (n = 10)	2	2	2	20	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
<i>C. felis</i> (n = 6)	1	1	1		2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
<i>C. meleagridis</i> (n = 2)	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>C. canis</i> (n = 1)	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cryptosporidium</i> sp. (n = 1)	1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Cystoisospora belli</i> (n = 5)	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cyclospora cayentanensis</i> (n = 5)	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Negative controls (n = 60 ^b)	0/20	1/20	0/20	1.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

^a The parasitology laboratories were Amiens University Hospital (Lab. A), Grenoble University Hospital (Lab. B), and Paris Saint-Louis Hospital (Lab. C).

^b Total, 20 negative controls per study center.

^c Not available in Lab. B for 1 sample (*C. felis* and *C. meleagridis*), 2 samples (*C. parvum*), or 3 samples (*C. hominis*).

negative controls. In Lab. B, one negative control, assessed by repeated microscopic examinations and confirmed to be negative by PCR (Lab. A), was positive by RIDAQuick. The specificity was 100% for Remel-Xpect, ImmunoCard STAT!, and Crypto-Strip and 98% for RIDAQuick. This confirms the high specificity of these tests, as previously reported in other studies performed on a larger number of samples (11, 13).

Analysis of the reliability of agreement between laboratories using Fleiss' kappa showed excellent interlaboratory reproducibility for RIDAQuick ($\kappa = 0.83$), Remel-Xpect ($\kappa = 0.80$), ImmunoCard STAT! ($\kappa = 0.84$), and Crypto-Strip ($\kappa = 0.78$). The sensitivity of *Cryptosporidium* antigen detection was significantly dependent on the kit and the species. The mean sensitivities for all *Cryptosporidium* species for all 3 laboratories were 47.2%, 62.4%, 68.8%, and 70.6% for Crypto-Strip, RIDAQuick, Remel-Xpect, and ImmunoCard STAT!, respectively. Since Crypto-Strip was significantly (chi-square test; $P = 0.04$) less sensitive than the other kits, analysis of the sensitivity according to the infecting species was performed exclusively on the results obtained with RIDAQuick, Remel Xpect, and ImmunoCard STAT! Data obtained with species other than *C. parvum* and *C. hominis* were pooled into a single group to increase sample size. No significant differences with respect to the sensitivity of detection of *C. parvum* (mean, 73.5%) and *C. hominis* (mean, 80.8%) (chi-square test; $P = 0.89$) were observed regardless of the immunoassay or the laboratory, while a lower sensitivity was observed for the other species (mean, 27.7%) (chi-square test; $P \leq 0.005$).

These results show that 3 of the 4 antigen detection tests presented similar sensitivities for the diagnosis of *C. parvum* or *C. hominis* infection, a relevant finding in countries in which *C. hominis* infections are highly endemic. The sensitivity for diagnosis of *C. parvum* or *C. hominis* infections was in the range of that reported by Johnston et al. (13) but lower than that claimed by the manufacturers or found in other studies (11, 14, 16). The possible effect of storage in potassium dichromate was examined in Lab. C by comparing the sensitivities of the four kits by the use of 4 groups of stool specimens containing *Cryptosporidium* oocysts (assessed by microscopy). The four specimen groups consisted of 20 fresh stool specimens collected prospectively, the same stool specimens stored in potassium dichromate for several days, 30 stool specimens stored in potassium dichromate for less than 12 months, and 30 stool specimens stored for 40 to 48 months (provided by the French ANOFEL network). We found excellent agreement between the results obtained with fresh and potassium dichromate-treated stools ($\kappa > 0.80$) and no significant effect of short-term storage (<12 months) or long-term storage (40 to 48 months) on the sensitivities of the immunoassays (chi-square or the Fisher exact test; $P > 0.3$ for all comparisons).

Despite good specificity, the limitation of all immunoassays tested in this study was their lower sensitivity for the diagnosis of infections due to *Cryptosporidium* species other than *C. parvum* or *C. hominis*. The consequences of this lower sensitivity should be limited in countries in which these species represent less than 5% of all *Cryptosporidium*-positive samples but should be considered in certain populations in which non-*C. parvum/C. hominis* species are more widely represented (3-5). In conclusion, these data show that some cases of cryptosporidiosis would have been missed if these assays had been

the only methods used for diagnosis. However, these tests could be useful when experienced stool microscopists are not available.

This study was supported by a grant from the Institut de Veille Sanitaire.

We thank Karine Guyot for her critical review of the manuscript, the members of the ANOFEL-*Cryptosporidium* National Network for providing clinical samples, and Anthony Saul for editing the manuscript.

We declare no conflict of interest in relation to this work.

REFERENCES

1. **Ajjampur, S. S., et al.** 2010. Multisite study of cryptosporidiosis in children with diarrhea in India. *J. Clin. Microbiol.* **48**:2075–2081.
2. **ANOFEL *Cryptosporidium* National Network.** 2010. Laboratory-based surveillance for *Cryptosporidium* in France, 2006–2009. *Euro Surveill.* **15**:pii:19642.
3. **Cacciò, S. M., and E. Pozio.** 2006. Advances in the epidemiology, diagnosis and treatment of cryptosporidiosis. *Expert Rev. Anti-Infect. Ther.* **4**:429–443.
4. **Cama, V. A., et al.** 2007. Differences in clinical manifestations among *Cryptosporidium* species and subtypes in HIV-infected persons. *J. Infect. Dis.* **196**:684–691.
5. **Cama, V. A., et al.** 2008. *Cryptosporidium* species and subtypes and clinical manifestations in children, Peru. *Emerg. Infect. Dis.* **14**:1567–1574.
6. **Chalmers, R. M., K. Elwin, A. L. Thomas, E. C. Guy, and B. Mason.** 2009. Long-term *Cryptosporidium* typing reveals the aetiology and species-specific epidemiology of human cryptosporidiosis in England and Wales, 2000 to 2003. *Euro. Surveill.* **14**:pii:19086.
7. **Chalmers, R. M., R. Smith, K. Elwin, F. A. Clifton-Hadley, and M. Giles.** 2010. Epidemiology of anthroponotic and zoonotic human cryptosporidiosis in England and Wales, 2004–2006. *Epidemiol. Infect.* **12**:1–13. [Epub ahead of print.]
8. **Chan, R., et al.** 2000. Evaluation of a combination rapid immunoassay for detection of *Giardia* and *Cryptosporidium* antigens. *J. Clin. Microbiol.* **38**:393–394.
9. **Garcia, L. S., and R. Y. Shimizu.** 1997. Evaluation of nine immunoassay kits (enzyme immunoassay and direct fluorescence) for the detection of *Giardia lamblia* and *Cryptosporidium parvum* in human fecal specimens. *J. Clin. Microbiol.* **35**:1526–1529.
10. **Garcia, L. S., and R. Y. Shimizu.** 2000. Detection of *Giardia lamblia* and *Cryptosporidium parvum* antigens in human fecal specimens using the ColorPAC combination rapid solid-phase qualitative immunochromatographic assay. *J. Clin. Microbiol.* **38**:1267–1268.
11. **Garcia, L. S., R. Y. Shimizu, S. Novak, M. Carroll, and F. Chan.** 2003. Commercial assay for detection of *Giardia lamblia* and *Cryptosporidium parvum* antigens in human fecal specimens by rapid solid-phase qualitative immunochromatography. *J. Clin. Microbiol.* **41**:209–212.
12. **Gatei, W., et al.** 2006. Cryptosporidiosis: prevalence, genotype analysis, and symptoms associated with infections in children in Kenya. *Am. J. Trop. Med. Hyg.* **75**:78–82.
13. **Johnston, S. P., M. M. Ballard, M. J. Beach, L. Causer, and P. P. Wilkins.** 2003. Evaluation of three commercial assays for detection of *Giardia* and *Cryptosporidium* organisms in fecal specimens. *J. Clin. Microbiol.* **41**:623–626.
14. **Llorente, M. T., et al.** 2002. Evaluation of an immunochromatographic dip-strip test for the detection of *Cryptosporidium* oocysts in stool specimens. *Eur. J. Clin. Microbiol. Infect. Dis.* **21**:624–625.
15. **Llorente, M. T., et al.** 2007. Genetic characterization of *Cryptosporidium* species from humans in Spain. *Parasitol. Int.* **56**:201–205.
16. **Weitzel, S., I. Dittrich, E. Möhl, E. Adusu, and T. Jelinek.** 2006. Evaluation of seven commercial antigen detection tests for *Giardia* and *Cryptosporidium* in stool samples. *Clin. Microbiol. Infect.* **12**:656–659.
17. **Wielinga, P. R., et al.** 2008. Molecular epidemiology of *Cryptosporidium* in humans and cattle in The Netherlands. *Int. J. Parasitol.* **38**:809–817.
18. **Xiao, L.** 2010. Molecular epidemiology of cryptosporidiosis: an update. *Exp. Parasitol.* **124**:80–89.