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

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**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); Immuno*Card STAT*! *Cryptosporidium*/*Giardia* rapid assay (Meridian Bioscience Inc.); Crypto-Strip (Coris BioConcept; (Belgium), and Remel-*X*pect *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-*X*pect and 6 with Immuno*Card 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

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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 | |
| *C. parvum* ($n$ = 20) | 15 | 15 | 14 | 73.3 | 15 | 14/18[c] | 14 | 74.1 | 16 | 15 | 13 | 73.3 | 9 | 10/18[c] | 11 | 50.1 |
| *C. hominis* ($n$ = 20) | 14 | 16 | 14 | 73.3 | 17 | 15 | 18 | 83.3 | 17 | 17 | 18 | 86.7 | 13 | 10/17[c] | 12 | 59.3 |
| Other *Cryptosporidium* species ($n$ = 10) | 2 | 2 | 2 | 20 | 3 | 3 | 3 | 30 | 3 | 4 | 3 | 33.3 | 1 | 1 | 1 | 10 |
| *C. felis* ($n$ = 6) | 1 | 1 | 1 | | 2 | 2 | 2 | | 2 | 3 | 2 | | 0 | 0/5[c] | 0 | |
| *C. meleagridis* ($n$ = 2) | 0 | 0 | 0 | | 0 | 0 | 0 | | 0 | 0 | 0 | | 0 | 0/1[c] | 0 | |
| *C. canis* ($n$ = 1) | 0 | 0 | 0 | | 0 | 0 | 0 | | 0 | 0 | 0 | | 0 | 0 | 0 | |
| *Cryptosporidium* sp. ($n$ = 1) | 1 | 1 | 1 | | 1 | 1 | 1 | | 1 | 1 | 1 | | 1 | 1 | 1 | |
| *Cystoisospora belli* ($n$ = 5) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Cyclospora cayetanensis* ($n$ = 5) | 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 |

[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-*X*pect, Immuno*Card 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-*X*pect ($\kappa$ = 0.80), Immuno*Card 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-*X*pect, and Immuno*Card 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 *X*pect, and Immuno*Card 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.

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