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Multicentric evaluation of a new real time PCR assay for Cryptosporidium spp.

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Background: *Cryptosporidium* spp. are a major cause of diarrheal disease in both immunocompetent and immunodeficient individuals. Cryptosporidium typically induces self-limiting diarrhoea. However, cryptosporidiosis may turn life-threatening in immunocompromised person, primarily those with AIDS. The majority of cryptosporidiosis cases in most countries are caused by *C. hominis* or *C. parvum*. Most of available diagnosis procedures are aimed at the above 2 species, however other species (*C. meleagridis, C. felis, C. canis, C. cuniculus*…) have also been detected in human stools. The aim of this study was to determine the effectiveness of a new commercial real-time PCR assay for the detection of 7 Cryptosporidium spp. in human stools.

Methods: In a multicentric study by 3 geographically distinct Hospital laboratory sites in France, the effectiveness of the real-time PCR (qPCR) Amplidiag Stool Parasite Kit (MOBIDIAG, Finland) for the detection of *Cryptosporidium* oocysts DNA in stools was assessed with 3 different qPCR devices (Rotorgene, CFX96 and ABI 7500 PCR system). In each participating laboratory, the following DNA extracts were obtained according to the manufacturer's instructions : (i) 10 DNA extracts from 200µL PBS or 200mg negative stools seeded with a parasite load of 500, 1000, 2500, 5000 or 50000 *C. parvum* oocysts per gram respectively, (ii) 40 DNA extracts from well characterized stool samples provided by the Crypto-Anofel network and selected to be representative of the diversity of *Cryptosporidium* species in France (*C. hominis, C. parvum, C. felis, C. cuniculus, C. canis, C. meleagridis, C. chipmunk*); (iii) 100 DNA extracts from human stools selected on the basis of negative microscopic results for *Cryptosporidium* spp. as negative controls, (iv) 3, 6 and 10 DNA extracts from human stool containing *Enterocytozoon bieneusi, Giardia intestinalis* and *Candida albicans*, respectively; for specificity assessment. In addition, 20 samples of unprocessed *Cryptosporidium* spp. positive stools were sent to the participating laboratories to test the qPCR using their own DNA extraction method.

Results: Of the 50 DNA from *Cryptosporidium* spp. positive samples, 3 were found qPCR-negative in 1/3 laboratories. A sample was found positive to *Cryptosporidium* in 1/3 laboratories among samples from microscopic *Cryptosporidium* oocyst negative stools and from *Enterocytozoon bieneusi, Giardia intestinalis* and *Candida albicans*, positive stools. The specificity of the test for *Cryptosporidium* spp. detection varied from 99.16% to 100%, and the sensitivity from 96% to 100%. High interlaboratory reproducibility was observed with no laboratory effect (ANOVA test: p=0.238). Twenty unprocessed *Cryptosporidium* spp. oocyst positive stools were qPCR-positive using both the manufacturer's and each participating laboratory's DNA extraction methods.

Conclusions: Data suggest that the present one-step qPCR assay which provides accurate extraction and detection of *Cryptosporidium* spp. DNA is well suited for the routine laboratory diagnosis of cryptosporidiosis with positive and negative predictive values of 99.32% and 99.16% respectively.