Multicentric Evaluation of a New Real-Time PCR Assay for Cryptosporidium spp.

R. RAZAKANDRAINIBE[1,2], E. FREALLE[3,4], C. NOURRISSON[5], S. VALOT[6], E. DUTOIT[3], L. FAVENNEC,[1,2], N. KAPEL[7], P. POIRIER[5], F. DALLE[6],' ANOFEL Cryptosporidium National Network

[1]Laboratoire de Parasitologie-Mycologie CHU-Rouen 1 Rue de Germont, 76000 Rouen

[2] EA3800 Protozooses transmises par l'alimentation Université de Rouen UFR Medicine et Pharmacie

[4]CHU Lille, Laboratoire de Parasitologie-Mycologie, F-59000 Lille, France

[4]Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 – UMR 8204 - CIIL - Center for Infection and Immunity of Lille, F-59000 Lille, France

[5]Laboratoire de Parasitologie Mycologie ; 58 rue Montalembert, CHU-Gabriel Montpied; 63003 Clermont-Ferrand Cedex1 France

[6]Laboratoire de Parasitologie-Mycologie Plateau Technique de Biologie du CHU 2 rue Angélique Ducoudray, BP 37013, 21070 Dijon Cedex

[7]Laboratoire de coprologie fonctionnelle, AP-HP, Hôpitaux Universitaires Pitié Salpêtrière-Charles Foix, Paris, France, EA 4065, Faculté de pharmacie, Université Paris Descartes, Paris, France.

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 small children, the elderly and immunocompromised person, primarily those with acquired immunodeficiency syndrome (AIDS). The majority of cryptosporidiosis cases in most countries are caused by *C. hominis* or *C. parvum.* Most of available detection kit are targeting and tested by using these two-major species, however other species (C. *meleagridis*, *C. felis*, *C*. canis, C. cuniculus…) and genotypes have been detected in human stools. The aim of this study was to determine the effectiveness of a detection kit toward 7 cryptosporidian species.

Methodology:

In a multicentric study by 3 geographically distinct Hospital laboratory sites in France (Clermont-Ferrand, Dijon, Lille), the effectiveness of the 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 Applied Biosystems 7500 PCR system).

For each participating team; (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 selected on the basis of negative microscopic results for *Cryptosporidium* as negative control, (iv) and for the specificity assessment 3 , 6 and 10 DNA extracts from human stool containing *Enterocytozoon bieneusi, Giardia intestinalis* and *Candida albicans* respectively; extracted following the manufacturer's instructions was provided. In addition, 20 samples of raw material were also sent to the participating lab to test the qPCR with their own specific DNA extraction method.

Results

Of the 50 DNA from *Cryptosporidium* positive samples, 3 samples were found negative in 1/3 laboratories. For each laboratory, the specificity is 100%, and the sensitivity varies from 96% to 100%. The interlaboratory variability was examined, ANOVA test showed no laboratory effect (p=0.238) attesting to the good reproducibility between laboratories. *Cryptosporidium* DNA extraction methods applied by each of the participating laboratories with the 20 raw positive samples does not affect the results, they were found *Cryptosporidium* spp PCR-positive.

Conclusion

To conclude, this one-step quantitative PCR is well suited to the routine diagnosis of cryptosporidiosis since practical conditions, including DNA extraction, have been positively assessed. With a positive and negative predictive value of 100% and 99.01% respectively, the kit provides accurate detection of *Cryptosporidium*