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Detection of *Cryptosporidium parvum* in human feces by PCR

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C. parvum **has a high pathologic potential also for man, especially for immunosuppressed patients. The microscopic detection of cysts in feces is neither easy nor always reliable. During the recent years, considerable progress has been achieved in establishing PCR-based approaches for i) sensitive detection of *C. parvum* in a variety of specimen types [3, 9-16, 18, 21, 24, 25, 27, 29-32, 34], ii) identification of individual genotypes of *C. parvum* [2, 4-7, 17, 19, 20, 22, 23, 26, 28, 33] and iii) viability testing of *C. parvum* organisms [8, 9, 13, 29, 30]. The protocols published so far include nested PCR [3, 8, 18, 34], RT-PCR [13, 29], and use of the UNG carryover prevention system [10]. The aim of this work was to establish a PCR system for the detection of *C. parvum* oocysts in stool samples, applying the same specimen preparation procedure as applied for imunofluorescence. In addition, we combined the UNG carryover prevention system with the use of long, PCR-generated digoxigenin-labelled probes, thus achieving a sensitivity comparable to nested PCR und circumventing the contamination risks associated with nested PCR protocols. We developed a simplified sucrose-cushion-based protocol for preparation of clinical specimens (adopted from [1]), satisfying both the needs of immunofluorescence and PCR.**

When tested with stool samples spiked with *C. parvum* oocysts, the analytical sensitivity of PCR was 3500 oocysts/ml stool (immunofluorescence: 3000 oocysts/ml stool), demonstrating that both methods were equivalent with respect to analytical sensitivity. However, when PCR and immunofluorescence were applied to clinical samples (n=5) with known positivity for *C. parvum*, only the specimen with the shortest duration of storage (5 weeks) could be correctly identified by PCR (clinical sensitivity: 20%). Our results demonstrate, that the PCR approach presented in this work is not suited for highly sensitive detection of *C. parvum* in faeces. This was mainly due to the fact that sucrose-gradient purified material was used, which relies on the presence of morphologically intact oocysts in the specimens. Desintegration of oocysts by excystation and/or storage may lower the parasite yield of the protocol drastically. As a consequence, a protocol extracting the entire DNA from faeces should be used for PCR for detection of *C. parvum* [34].

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