Detection of *Cryptosporidium* and *Giardia* in clinical laboratories in Europe—a comparative study

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Abstract

To determine the routine diagnostic methods used and compare the performance in detection of oocysts of *Cryptosporidium* species and cysts of *Giardia intestinalis* in faecal samples by European specialist parasitology laboratories and European clinical laboratories. Two sets of seven formalin-preserved faecal samples, one containing cysts of *Giardia intestinalis* and the other, containing oocysts of *Cryptosporidium,* were sent to 18 laboratories. Participants were asked to examine the specimens using their routine protocol for detecting these parasites and state the method(s) used. Eighteen laboratories answered the questionnaire. For detection of *Giardia*, 16 of them used sedimentation/concentration followed by light microscopy. Using this technique the lower limit of detection of *Giardia* was 17.2 cysts/mL of faeces in the best performing laboratories. Only three of 16 laboratories used fluorescent-conjugated antibody-based microscopy. For detection of *Cryptosporidium* acid-fast staining was used by 14 of the 17 laboratories that examined the samples. With this technique the lower limit of detection was 976 oocysts/mL of faeces. Fluorescent-conjugated antibody-based microscopy was used by only five of the 17 laboratories. There was variation in the lower limit of detection of cysts of *Giardia* and oocysts of *Cryptosporidium* between laboratories using the same basic microscopic methods. Fluorescent-conjugated antibody-based microscopy was not superior to light microscopy under the conditions of this study. There is a need for a larger-scale multi-site comparison of the methods used for the diagnosis of these parasites and the development of a Europe-wide laboratory protocol based upon its findings.

Keywords: *Cryptosporidium*, direct fluorescent-antibody tests, enzyme immunoassay, formalin-ethyl acetate faecal concentrate, *Giardia intestinalis*, modified Ziehl–Neelsen, oocysts
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Introduction

Giardia intestinalis and *Cryptosporidium* spp. are parasitic protozoa of cosmopolitan distribution. Transmitted by the ingestion of cysts or oocysts, respectively, in contaminated food or water, they are major sources of diarrhoeal disease in humans. They are reported to account for 23–32% of protozoa-related drinking water outbreaks worldwide [1] and the impact of climate change is likely to increase the risk of future water-borne outbreaks of both these parasites [2].

Various methods are available for the laboratory detection of protozoan parasites in faecal samples. The characteristic cysts of *G. intestinalis* measuring 8–12 μm can be found by examination of the deposit of a formalin–ether or formalin–ethyl acetate faecal concentrate preparation [3]. Unlike the cysts of *Giardia*, the oocysts of *Cryptosporidium*, measuring 4–6 μm in diameter, do not concentrate well using standard concentration techniques but can be identified by microscopy combined with various staining methods, i.e. modified Ziehl–Neelsen or phenol–auramine-based fluorescence microscopy of faecal smears [4]. However, because cyst or oocyst excretion can be sporadic, these diagnostic stages may be

missed if only one sample is examined, so three consecutive specimens are commonly requested to increase the chance of detection. Specific antigen detection methods are also deployed to diagnose *Giardia* and *Cryptosporidium* infection and these include direct fluorescent-antibody tests, ELISA and immunochromatographic cartridge assays. Some kit manufacturers have combined the detection of both these parasites into a single kit or test device. Both parasite species can also be detected in faecal samples using molecular methods.

The choice of methods to detect these protozoa varies between different clinical laboratories. Therefore, the European Society of Clinical Microbiology and Infectious Diseases Study Group on Clinical Parasitology in collaboration with the UK National External Quality Assurance Service (UK NEQAS) for Parasitology undertook this study to determine the routine diagnostic tests deployed and evaluate the sensitivity of the different techniques used for the detection of oocysts of *Cryptosporidium* species and cysts of *Giardia* in faecal samples.

Materials and Methods

Twenty-two European laboratories were invited to participate in the study. All the laboratories approached take part in the UK NEQAS Faecal Parasitology Scheme.

An initial questionnaire was sent to those laboratories to ascertain the methods that they used to examine for these parasites in clinical samples. Eighteen laboratories, three from the UK, two from each of Germany, Norway and Portugal and one from each of Austria, Croatia, Greece, Italy, the Netherlands, Romania, Slovenia, Sweden and Switzerland accepted the invitation to participate in the study, 11 were specialist parasitology laboratories and seven were clinical microbiology laboratories but all could be considered to be relatively experienced in the field.

Two distributions, each consisting of seven faecal samples preserved in formalin, were sent to those laboratories. Each sample contained 1 mL of faeces equivalent to 1 g because this is the recommended size of faecal sample for use in concentration methods. One distribution contained cysts of *G. intestinalis* and the other contained oocysts of *C. parvum*. Participants were asked to examine one set of samples for oocysts of *Cryptosporidium* and the other set of samples for cysts of *Giardia* using their routine protocol in each case. All samples were coded so that study participants could not know whether they might be positive.

Giardia intestinalis

The specimen containing cysts of *G. intestinalis* consisted of a formalin-fixed faecal sample. To evaluate the number of cysts present, the sample was blended using a processor to ensure homogeneous distribution of the cysts throughout the specimen. A 20-μL sample of specimen was placed on a microscope slide, a coverslip was applied and the total area of a 22 × 22-mm cover slip was examined and the number of *Giardia* cysts was counted. Using this figure, the number of cysts per millilitre was found to be 172 000. Five ten-fold dilutions were made with parasite-negative faeces and tested in the Department of Clinical Parasitology before distribution by microscopy following concentration by the Parasep faecal concentrator, by MERIFLUOR C/G (Meridian Life Science, Inc., Memphis, TN, USA) (an *in vitro* direct fluorescent-antibody procedure; Meridian Bioscience, Cincinnati, OH, USA) and ImmunoCard STAT! (Meridian Life Science, Inc.) (an immunochromatographic assay; Meridian Bioscience). All commercial tests were performed according to the manufacturer's instructions. The pre-distribution results are shown in Table 1.

Cryptosporidium parvum

The specimen containing oocysts of *C. parvum* was purchased from a commercial company and had an initial concentration of

Specimen no.	Concentration. of cysts/oocysts/mL pre-formalin ethyl acetate concentration	No. of cysts/oocysts/coverslip post-formalin ethyl acetate concentration	MERIFLUOR	ImmunoSTAT!
1	172 000	>1 000	+++	Positive
2	Negative	Negative	Negative	Negative
3	172	6	+	Negative
4	17 200	750	+++	Positive (weak)
5	Negative	Negative	Negative	Negative
6	17.2	Negative	Negative	Negative
7	1 720	175	+	Negative
A	62 500	39	+++	Weak positive
B	976	1	–	Negative
C	15 625	18	+++	Negative
D	Negative	0	–	Negative
E	7812	12	+++	Negative
F	1953	1	+	Negative
G	3906	3	+	Negative

TABLE 1. Concentration of cysts of *Giardia intestinalis* and oocysts of *Cryptosporidium parvum* per millilitre pre-concentration and the number of cysts/oocysts per coverslip post-concentration. The oocysts of *Cryptosporidium parvum* were stained with modified Ziehl–Neelsen post-concentration Specimens 1–7 *Giardia intestinalis*; Specimens A–G *Cryptosporidium parvum*