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*

100 million oocysts per millilitre. This was diluted in 10% formalin to give a concentration of one million oocysts per millilitre. The resulting suspension was added to an equal volume of liquid faeces, preserved in 10% formalin, which was negative for parasites. A number of different dilutions were made and tested in the Department of Clinical Parasitology before distribution using microscopy following concentration by the Parasep faecal concentrator and staining by the modified Ziehl–Neelsen method [3]; by MERIFLUOR C/G and ImmunoCard STAT!. All tests were performed according to the manufacturer's instructions.

Partly as a result of postal problems beyond the laboratory's control, only 12 participants responded to the initial survey. As a result of this and also because the concentration of *Cryptosporidium* oocysts present in the specimens was high with few reported negatives, making it impossible to determine the analytical sensitivity, a second distribution containing a lower concentration of oocysts was sent out. The pre-distribution results are shown in Table 1.

Results

Of the 18 participants who agreed to take part in the study, 16 returned results for *Giardia* and 17 returned results for *Cryptosporidium*. The methods used by participants to detect cysts of *Giardia* are shown in Table 2 and methods used for oocysts of *Cryptosporidium* are shown in Table 3. The results obtained by participants for microscopy are shown in Table 4 for *Giardia* and Table 5 for *Cryptosporidium*. As the specimens were formalin-fixed, only the results from methods that can be used on such specimens are included. Hence, data from some immunochromatographic tests and all PCR tests are not shown for either parasite, because

TABLE 2. Methods used by 16 participants for the detection of cysts of *Giardia intestinalis*. Some used more than one method

Methods used	Kit	No. using the test	Appropriate specimens for testing
Light microscopy methods	Sedimentation/ concentration method	14	Fresh faeces or preserved in 10% formalin
	Sucrose Gradient Concentration	1	Fresh faeces or preserved in 10% formalin
Fluorescence-conjugated antibody-based microscopy	Aqua Glo GL[a]	2	Water particulates. Excluded from analysis
	Meridian Bioscience[b] (MERIFLUOR)	1	Fresh faeces, preserved in 10% formalin or SAF
Immunoassay for antigen	TechLab Giardia[c]	3	Fresh faeces or preserved in 10% formalin
	Xpect Giardia (Remel)[d]	2	Fresh faeces, preserved in 10% formalin or SAF
	Triage Biosite[a]	1	Fresh, unfixed faeces. Excluded from analysis
	Rida screen Giardia (Biopharm)[e]	2	Fresh, unfixed faeces. Excluded from analysis
	IVD Research Inc. ELISA	1	Fresh faeces, preserved in 10% formalin or SAF
PCR	In-house	1	Fresh, unfixed faeces. Excluded from analysis

SAF, sodium acetate-acetic acid formalin.
[a]WaterborneTM Inc, Clinical and Environmental Parasitology Products, New Orleans, LA, USA.
[b]Memphis, TN, USA.
[c]Blacksburg, VA, USA.
[d]Thermo Fisher Scientific, Lenexa, KS, USA
[e]An der neuen Bergstraße 17, Darmstadt, Germany.

TABLE 3. Methods used by 17 participants for the detection of oocysts of *Cryptosporidium parvum*. Some used more than one method

Method used	Kit	No. using the test	Appropriate specimens for testing
Microscopy plus staining	Modified Ziehl–Neelsen	14	Fresh faeces, faeces preserved in 10% formalin or methanol fixed slides
	Phenol auramine	2	Fresh faeces, faeces preserved in 10% formalin or methanol fixed slides
	Kinyoun stain	1	Fresh faeces, faeces preserved in 10% formalin or methanol fixed slides
	Waterborne aqua-glo[a]	1	Water particulates. Excluded from analysis
Fluorescence-conjugated antibody-based microscopy	Waterborne direct immunofluorescence.	2	Water particulates. Excluded from analysis
	Bios GMBH[b]	1	Fresh faeces, faeces preserved in 10% formalin or SAF
	Meridian Bioscience (Merifluor)	1	Fresh faeces or faeces preserved in 10% formalin
Antigen detection test	r-Biopharm	1	Fresh unpreserved faeces. Excluded from analysis
	Triage Microparasite Panel Biosite	1	Fresh unpreserved faeces. Excluded from analysis
PCR	In-house	1	Fresh unpreserved faeces. Excluded from analysis
	EasyMag[b]	1	Fresh unpreserved faeces. Excluded from analysis

SAF, sodium acetate-acetic acid formalin.
[a]Clinical and Environmental Parasitology Products, New Orleans, LA, USA.
[b]bioMerieux, INSTITUT MÉRIEUX, Lyon, France.

TABLE 4. Performance in detection of *Giardia intestinalis* cysts

	No. of participants achieving positive test results		
	Concentration followed by microscopy *n* = 15		
No of cysts/mL	Reference (*n* = 8)	Non-reference (*n* = 7)	Total
0	0	0	0
0	0	1 False positive (+2 equivocal)	1 False positive
17.2	0	2 (+1 equivocal) (29%)	2 (13%)
172	2 (+1 equivocal) (25%)	4 (57%)	6 (40%)
1720	8 (100%)	6 (86%)	14 (93%)
17 200	8 (100%)	7 (100%)	15 (100%)
172 000	8 (100%)	7 (100%)	15 (100%)

fresh unfixed faecal samples are preferred for those kits and for PCR.

Detection of *Giardia* cysts

Eight laboratories used more than one test. Four laboratories gave overall results for each organism as opposed to individual results by test; two laboratories used a fluorescent-antibody-based antigen detection test alone; one laboratory used an antigen detection test alone; one laboratory stated that their routine was to examine the samples with PCR in conjunction with other tests. The specialist laboratories used a wider range of tests, i.e. concentration techniques, fluorescent-conjugated antibody-based microscopy and immunoassays for antigens, than the non-specialist laboratories, which used mainly concentration techniques with one using a fluorescent-conjugated antibody-based microscopy method.

The lowest limit of cyst detection for the non-reference laboratories was 17.2 cysts/mL and was achieved by only 2/16 (12.5%) laboratories using concentration followed by microscopy compared with 172 cysts/mL achieved by 2/16 (12.5%) reference laboratories using this method. (Table 4) Comparison of this method with antigen detection tests (used by reference laboratories only) and fluorescence-based microscopy (used by both reference and non-reference laboratories) showed that the fluorescent-antibody-based antigen detection tests performed almost as well, detecting 172 cysts/mL. Performance was similar in reference versus non-reference laboratories at high cyst numbers but there was a trend to better performance by non-reference laboratories in detecting lower cyst numbers (Table 4).

Detection of *Cryptosporidium* oocysts

Nine laboratories reported the use of more than one test; six laboratories used modified Ziehl–Neelsen alone; one used a fluorescence-based antigen detection test alone; two laboratories used PCR in conjunction with other tests. The specialist parasitology laboratories used a wider range of tests, i.e. modified Ziehl–Neelsen, phenol–auramine and Kinyoun stains followed by microscopy, fluorescent-conjugated antibody-based microscopy and antigen detection tests, than the non-specialist laboratories, with the latter using mainly modified Ziehl–Neelsen stain followed by microscopy with one using a fluorescent-conjugated antibody-based microscopy. Modified Ziehl–Neelsen staining followed by microscopy had the lowest limit of oocyst detection (976 oocysts/mL) but it was only reached in one of 15 laboratories using that method (Table 5). Using modified Ziehl–Neelsen staining, although the specialist laboratories were able to detect a lower number of oocysts of *Cryptosporidium* than the non-specialist laboratories (976 oocysts/mL versus 3906 oocysts/mL, respectively), this was only achieved by one laboratory at each of 1953 and 976 oocysts/mL. However, overall the non-reference laboratories had higher detection rates than the reference laboratories.

The fluorescent-antibody-based microscopy tests did not perform better than modified Ziehl–Neelsen staining or auramine-based fluorescence (Table 5). It was not possible to compare results with PCR because this test requires unfixed faecal samples.

Discussion

The aim of this study was to establish the methods used by a sample of European diagnostic laboratories; determine which method detected the lowest concentration of parasites (i.e.

TABLE 5. Method performance in detection of oocysts of *Cryptosporidium parvum* expressed as number of positive tests

	Stain followed by microscopy *n* = 15				
No. of oocysts/mL	Ref. labs *n* = 7	Non-ref. labs *n* = 8	Total	Phenol auramine (ref. labs only) *n* = 2	Immunofluorescent test (non-ref. labs only) *n* = 2
0	0	2 False positives	2 Fp	0	0
976	1 (14%)	0 (+1 Equivocal)	1 (7%)	0	0
1953	1 (14%)	0 (+1 Equivocal)	1 (7%)	0	0
3906	5 (71%)	8 (100%)	13 (87%)	2	1
7812	4 (57%)	5 (63%)	9 (60%)	1	0 (+1 Equivocal)
15 625	3 (+1 Equivocal) (43%)	5 (+1 Equivocal) (63%)	8 (53%)	2	1
62 500	4 (57%)	8 (100%)	12 (80%)	2	1

had the greatest analytical sensitivity) and see if there was a difference in level of detection achieved by specialist parasitology laboratories and non-specialist clinical laboratories. The specimens distributed were treated with formalin to ensure that their morphological integrity was maintained through the process of specimen preparation, distribution and examination in the receiving laboratories. This necessary step prevented the assessment of some antigen detection kits and PCR, because some antigen detection kits and PCR methods are adversely affected by the presence of formalin and require fresh, unfixed faecal samples for optimum performance. Although it was not possible to supply such material to participating laboratories in this study, future Parasitology EQA schemes will need to take account of this. For example, if there was sufficient demand, a future EQA distribution could include fresh unfixed samples shipped in PCR extraction buffer. Microscopy methods require expertise in identifying parasites and it has been shown that clinical laboratory personnel have difficulty in some cases in distinguishing artefacts from the parasite stages present in faecal samples [5]. Indeed, in this study, one and two laboratories, respectively, reported parasites in the specimens that were negative for cysts of *Giardia* and oocysts of *Cryptosporidium*. As would be expected for both *Giardia* and *Cryptosporidium*, the higher the concentration of cysts or oocysts in the specimen, the more likely they were to be detected by microscopic methods. However, some participants failed to detect cryptosporidial oocysts in specimens with relatively high concentrations present (Table 5).

Transposition errors in reporting the results may have occurred in some cases, because those laboratories that reported parasites in the negative specimens reported no parasites in some of the positive samples.

The specialist parasitology laboratories used a wider range of tests than the clinical laboratories. This is not surprising, as the specialist laboratories are also likely to be reference centres.

The method most commonly used for the detection of *Giardia* cysts in the laboratories surveyed was a concentration method followed by microscopy and for *Cryptosporidium* oocysts a staining method followed by microscopy. Antigen detection kits to diagnose these parasites, separately or in combination, were also deployed although it was notable that the non-specialist laboratories used mainly concentration techniques for the detection of *Giardia* cysts and modified Ziehl–Neelsen-stained faecal smears for the detection of *Cryptosporidium* oocysts.

Specialist laboratories are more likely to be staffed by personnel with substantial expertise in parasitology. In this study, specialist laboratories were able to detect oocysts of *Cryptosporidium* present at lower numbers than could be detected by general laboratories, but the reverse was true for the detection of *Giardia* cysts. All laboratories in this study were enrolled in the UK NEQAS Faecal Parasitology scheme and it is likely that this and other parasitology EQA schemes deployed in Europe over the last few decades have contributed to improved standards [5], although there is still more work required. It must also be noted that the study participants knew they were looking for *Giardia* in one set or *Cryptosporidium* in the other set of specimens, so the results are likely to be close to their best performance, though it is possible that a suspected *Giardia* cyst or *Cryptosporidium* oocyst might be less likely to be dismissed as an artefact under the conditions of this study than in a clinical specimen and this might have contributed to the false positives encountered.

Unlike many of the reports comparing diagnostic performance for these parasites, this was a multi-centre study, but as the number using each particular diagnostic kit was small, meaningful statistical analysis comparing the various products was not feasible. However, it is still possible to see that for *Giardia* cyst and *Cryptosporidium* oocyst detection, fluorescent-antibody-based microscopy did not perform better than light microscopy. In contrast, Garcia *et al.* [6] reported better sensitivity using direct immunofluorescence microscopy for both of these parasites. Tee *et al.* [7] found the Giardia CEL IF test (TCS Biosciences Ltd, Botolph Claydon, Buckingham, UK) to be more sensitive than microscopy of a formalin–ether faecal concentrate and the Crypto CEL IF test to be more sensitive than phenol–auramine-based fluorescence microscopy, which was itself more sensitive than acid-fast staining for the detection of *Cryptosporidium*. Kehl *et al.* [8] reported high sensitivity (96%) for *Cryptosporidium* with the MERIFLUOR kit but reported the same sensitivity for acid-fast staining. Johnston *et al.* [9] found the MERIFLUOR direct fluorescent-antibody test to be more sensitive than the ImmunoCard STAT! and the ProSpecT EZ microplate assay for *Giardia* and for detection of *Cryptosporidium*, the MERIFLUOR direct fluorescent-antibody test was more sensitive than the ProSpecT microplate assay, the ImmunoCard STAT! test and acid-fast stained faecal smears, in all cases testing faecal samples that had been preserved in 10% formalin. Chalmers *et al.* [10] reported superiority of fluorescent-antibody-based microscopy for detection of *Cryptosporidium*, Crypto-Cel IFM achieving 97.4% sensitivity, compared with 92.1% for phenol–auramine-based fluorescence and 75.7% for modified Ziehl–Neelsen staining.

In contrast to other studies or indeed EQA schemes, where clinical samples are tested on a positive or negative basis, the present study examined faecal samples for which the number of cysts or oocysts per gram was known and sought an end-point, so it was possible to determine the lower limit of

detection or analytical sensitivity for the tests. For *Giardia* pre-distribution, microscopy plus iodine detected cysts in the sample containing 172 cysts/mL, but failed to do so at 17.2 cysts/mL. Post-distribution, this technique detected cysts at 17.2/mL, but in only two of 18 laboratories using it, so although a given technique has an intrinsic lower limit of detection, it may not be reached operationally by all users. Table 4 further illustrates this point; in the best performing laboratories, cyst concentrations as low as 17.2/mL were detected, yet detection of *Giardia* cysts was not achieved by all laboratories until a concentration of 17 200 cysts/mL was present. For *Cryptosporidium* the lower limit of detection both pre-distribution and post-distribution was 976 cysts/mL for concentration followed by modified Ziehl–Neelsen staining. For MERIFLUOR the limit was 1953 cysts/mL pre-distribution and 3906 cysts/mL post-distribution. There is little information from studies other than this one on test performance versus cysts or oocysts per gram of faeces, as many of them used clinical samples with a single established test or a composite standard of two tests as the reference standard. For example, in a study of commercial assays for *Giardia* and *Cryptosporidium* detection, Johnston *et al.* [9] used the MERIFLUOR test to calculate sensitivity and specificity for the comparator tests. Although they used cyst numbers to help rank tests, they were based on the number of cysts or oocysts seen by MERIFLUOR per drop (approximately 10 μL) of sediment obtained after formalin–ethyl acetate stool concentration rather than cysts or oocysts per gram of pre-concentration stool. Quantitative cyst and oocyst counts are regularly measured to assess drinking water safety [1] and very sensitive methods have been developed, such as immunomagnetic bead separation, which has a limit of detection of two cysts or oocysts per gram of bovine faeces. Using this method in rural Canada, Lalancette *et al.* [1] reported median concentrations of 111 *Giardia* cysts/g of calf faeces (maximum 1 939 333 cysts/g) and 333 *Cryptosporidium* oocysts/g of calf faeces (maximum 44 607 oocysts/g). The best-performing test in the best-performing laboratory in the present study detected 17.2 *Giardia* cysts/mL (gram) of stool so would have detected a good many of the bovine infections reported by Lalancette *et al.* [1], but the *Cryptosporidium* tests, with the best performer detecting 976 cysts/mL, would have missed many cases of cryptosporidiosis. Unfortunately, although very sensitive, the immunomagnetic bead separation technique is too cumbersome for use on large numbers of clinical samples. Weber *et al.* [11] found a lower limit of detection of 5000 *Cryptosporidium* oocysts per gram of seeded watery diarrhoeal stool specimens for both MERIFLUOR and acid-fast staining, though MERIFLUOR was positive in more of the samples (9/10 versus 6/10). Few of the laboratories in the present study used antibody-based fluorescence microscopy to detect *Giardia* or *Cryptosporidium* despite many authorities regarding it as the microscopic method of choice. This may be because it cannot be automated, or because it does not remove the need for faecal concentration to detect helminths or indeed protozoa. PCR is clearly more sensitive than microscopy. For example, Miller and Sterling described successful detection of a single *Giardia* cyst using nested PCR [12]. In the future, multiplex real-time PCR is likely to become the method of choice for the detection of *Giardia* and *Cryptosporidium* in clinical laboratories. The clinical and epidemiological need for this is clear. For example, during 2011 there was an outbreak of cryptosporidiosis in a town in northern Sweden. In all, 155 cases were diagnosed and more than 6000 persons had symptoms. The drinking water had to be boiled for months. The diagnosis was not made until a laboratory technician identified *Cryptosporidium* in a routine sample (B. Evengard; personal communication, 2013). Had multiplex PCR been in use the diagnosis would have been secured earlier in this large outbreak. Primary health-care clinicians rarely ask for specific diagnostics for this parasite, but routine application of multiplex PCR for faecal pathogens in cases of diarrhoea would address that.

Although only one laboratory in the current survey used PCR, at least one other laboratory that took part has introduced PCR for the diagnosis of both these parasites. EQA schemes will need to adapt to support these assays as they become widely used.

Conclusions

There is variation in the lower limit of detection of cysts of *Giardia* and oocysts of *Cryptosporidium* between laboratories using microscopic methods. Surprisingly few laboratories used antibody-based fluorescence microscopy to detect them. There is a need for larger-scale comparison of the methods in use and for more detailed information on the sensitivity achieved in practice. Development of a Europe-wide laboratory protocol should then be planned. As PCR becomes more widely used in routine laboratories, Parasitology EQA schemes will need to supply alternative specimens to the formalin-fixed faecal samples currently distributed.

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