

Parasitology: United Kingdom National Quality Assessment Scheme

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Abstract

Aims: To assess the results from parasitology laboratories taking part in a quality assessment scheme between 1986 and 1991; and to compare performance with repeat specimens.

Methods: Quality assessment of blood parasitology, including tissue parasites (n = 444; 358 UK, 86 overseas), and faecal parasitology, including extra-intestinal parasites (n = 205; 141 UK, 64 overseas), was performed.

Results: Overall, the standard of performance was poor. A questionnaire distributed to participants showed that a wide range of methods was used, some of which were considered inadequate to achieve reliable results. Teaching material was distributed to participants from time to time in an attempt to improve standards. **Conclusions:** Since the closure of the IMLS fellowship course in 1972, fewer opportunities for specialised training in parasitology are available: more training is needed. Poor performance in the detection of malarial parasites is mainly attributable to incorrect speciation, misidentification, and lack of equipment such as an eyepiece graticule.

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The United Kingdom National External Quality Assessment Scheme for Microbiology (NEQAS), which covers many areas of microbiology, has already been described.^{1,2} As part of this scheme, simulated specimens are despatched to participants, who examine them and report their results to the Quality Assurance Laboratory (QAL). Before 1986 specimens containing parasites were occasionally distributed as part of the general bacteriology quality assessment scheme, there being no separate subscheme for parasitology. Several factors, including retirement of pathology staff who had gained considerable overseas experience in former years, allied to diminished overseas experience of trainee pathologists and closure of the IMLS fellowship course in parasitology in 1972, has led to the gradual decline in the standard of diagnostic parasitology in the United Kingdom. This trend was noted through results from earlier NEQAS distributions, discussions with laboratory staff around the country, an increase in misdiagnoses and more routine work being referred to the Hospital for Tropical Diseases, London

(HTD). The parasitology of the immunocompromised is also becoming increasingly important and not all laboratories may be prepared to tackle rare opportunistic infections.

To help remedy these deficiencies, in 1986 QAL (then part of the (Division of Microbiological Reagents and Quality DMRQC) together with the Department of Clinical Parasitology, HTD, began to develop a quality assessment control) scheme for parasitology. Two subschemes were established: blood parasitology, including tissue parasites and faecal parasitology, including extra-intestinal parasites. Objectives of the parasitology schemes were to provide participants with a variety of specimen material to increase awareness and skills in this specialty, and to educate participants through the production of teaching sheets describing unfamiliar parasites and containing recommendations for standardised methods.

To facilitate access to specimen material and specialised expertise, the day to day functions of the scheme were located at HTD with administrative support based at QAL. Distributions began on a regular basis in January 1987. Since June 1991, NEQAS parasitology has also regularly supplied blood films for distribution by the NEQAS haematology scheme to haematology departments.

Methods

PARTICIPANTS

A total of 444 laboratories participate in the faecal parasitology scheme of which 358 are in the United Kingdom and 86 are overseas. In the blood parasitology scheme 205 laboratories participate, 141 from the United Kingdom and 64 from overseas.

The parasites distributed are shown in tables 1 to 4. Most material distributed was obtained from patients with known infections and was selected on the basis of the presence of adequate numbers of parasites, with characteristic morphological features and sufficient quantities of specimen. Where naturally infected material was not available, alternative sources were used. *Trichomonas vaginalis* trophozoites were cultured in TY1-S-33 medium³ from which smears were prepared, dried, fixed in methanol and distributed unstained. *Giardia lamblia* trophozoites were cultured similarly, fixed in formalin, and dispensed into phials before distribution as a simulated jejunal fluid. Filariform larvae of Hookworm spp were obtained by the Harada-Mori filter paper strip culture^{4,5} and *Strongyloides stercoralis* by char-

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Table 1 Single blood and tissue parasites distributed and participants' results

Specimen number	Species	Number of laboratories	Correct genus species, and stage	Correct genus only	Incorrect species or stage	Unexpected parasite only	None found	Multiple parasites, genera, species or stages
1336	<i>Plasmodium vivax</i>	80	44 (55.0%)	9 (11.2%)	15 (18.8%)			12 (15.0%)
1686	<i>Plasmodium vivax</i>	116	91 (78.4%)	7 (6.0%)	9 (7.8%)			9 (7.8%)
1917	<i>Plasmodium vivax</i>	119	93 (78.2%)	3 (2.5%)	5 (4.2%)	1 (0.8%)		17 (14.3%)
2256	<i>Plasmodium vivax</i>	138	102 (73.9%)	5 (3.6%)	17 (12.3%)		2 (1.5%)	12 (8.7%)
1435	<i>Plasmodium malariae</i>	83	55 (66.3%)	10 (12.0%)	14 (16.9%)		3 (3.6%)	1 (1.2%)
1957	<i>Plasmodium malariae</i>	113	81 (71.7%)	8 (7.0%)	13 (11.5%)	7 (6.2%)	3 (2.7%)	1 (0.9%)
2178	<i>Plasmodium malariae</i>	137	99 (72.3%)	8 (5.8%)	18 (13.1%)	1 (0.7%)	9 (6.6%)	2 (1.5%)
1628	<i>Plasmodium ovale</i>	111	59 (53.2%)	17 (15.3%)	31 (27.9%)	1 (0.9%)	1 (0.9%)	2 (1.8%)
1384	<i>Plasmodium falciparum</i> *	84	66 (78.6%)	7 (8.3%)	6 (7.1%)			5 (6.0%)
2363	<i>Plasmodium falciparum</i> *	127	90 (70.9%)	—	1 (0.8%)			36 (28.3%)
1516	<i>Trypanosoma brucei</i> sp	103	40 (38.8%)	61 (59.2%)	2 (2.0%)			
1976	<i>Trypanosoma brucei</i> sp	125	91 (72.8%)	20 (16.0%)	10 (8.0%)	1 (0.8%)	2 (1.6%)	1 (0.8%)
1977	<i>Trypanosoma cruzi</i>	125	97 (77.6%)	18 (14.4%)	8 (6.4%)			2 (1.6%)
2201	<i>Leishmania</i> sp	134	124 (92.6%)	1 (0.7%)		3 (2.2%)	5 (3.8%)	1 (0.7%)
1575	<i>Loa loa</i>	110	54 (49.1%)	25† (22.7%)		23** (20.9%)	8 (7.3%)	
1847	<i>Loa loa</i>	117	97 (82.9%)	8† (6.8%)		12** (10.3%)		
2151	<i>Loa loa</i>	133	72 (54.1%)	15† (11.3%)		36** (27.1%)	10 (7.5%)	
2370	<i>Onchocerca volvulus</i>	122	79 (64.8%)	10† (8.2%)	3 (2.4%)	24** (19.7%)		6 (4.9%)

*For identification only, not assessment of parasitaemia.

** Includes incorrect identification of *Microfilariae*.

†The reporting of "*Microfilariae* present" has been considered equivalent to genus level identification.

Specimen 2285 was a mixed sample containing *Plasmodium falciparum* and *Plasmodium vivax*. The following results were obtained:

18/140 laboratories (12.9%) reported correct genus, species, and stage; two of 140 laboratories (1.4%) reported correct genus only; nine of 140 laboratories (6.4%) reported incorrect species or stage; and 111 of 140 laboratories (79.3%) reported the presence of a single species only.

Two negative samples (no parasites present) were distributed and the following results were obtained:

specimen 1763: 93/96 laboratories (96.9%) reported no parasites present; three of 93 laboratories (3.1%) reported an unexpected parasite.

specimen 2344: 134/139 laboratories (96.4%) reported no parasites present; five of 139 laboratories (3.6%) reported an unexpected parasite.

Table 2 Percentage parasitaemias of *Plasmodium falciparum* and participants' results

Specimen number	Actual % parasitaemia	Number of laboratories (total examining)	% parasitaemia reported	Number of laboratories reporting result
1956	< 1%	107	< 2%	105 (98.0%)
			5–10%	2 (2.0%)
			< 4.5%	1 (0.9%)
			4.5–8.5%	41 (37.6%)
			8.6–10.0%	37 (33.9%)
1738	6.5%	109	10.1–12.0%	7 (6.4%)
			12.0–15.0%	16 (14.7%)
			15.1–20.0%	4 (3.7%)
			> 20%	3 (2.8%)
			9–11%	1 (0.8%)
			15–17%	2 (1.6%)
			18–20%	7 (5.5%)
			21–25%	42 (33.1%)
			26–28%	24 (18.9%)
			29–31%	23 (18.1%)
2323	23%	127	32–34%	7 (5.5%)
			35–37%	6 (4.7%)
			38–40%	10 (7.9%)
			> 40%	5 (3.9%)

Table 3 Single faecal parasites distributed and participants' results

Specimen number	Species	Number of laboratories	Correct genus species, and stage	Correct genus only	Incorrect species, stage, or partial identification	Unexpected parasite only	None found	Multiple parasites, genera, species or stages
Nematodes:								
1383	<i>Ascaris lumbricoides</i>	338	258 (76.3%)			9 (2.7%)	63 (18.6%)	8 (2.4%)
1599	<i>Ascaris lumbricoides</i>	340	314 (92.3%)			5 (1.5%)	15 (4.4%)	6 (1.8%)
1710	<i>Ascaris lumbricoides</i>	343	333 (97.1%)		1 (0.3%)	1 (0.3%)	2 (0.6%)	6 (1.7%)
1955	<i>Ascaris lumbricoides</i>	330	308 (93.3%)			5 (1.5%)	15 (4.6%)	2 (0.6%)
1626	Hookworm sp	341	325 (95.3%)			6 (1.8%)	8 (2.3%)	2 (0.6%)
2368	Hookworm sp	343	285 (83.1%)		3 (0.9%)	6 (1.7%)	23 (6.7%)	26 (7.6%)
1737	Hookworm sp (filariform larvae)	342	180 (52.6%)		17 (5.0%)	130† (38.0%)	1 (0.3%)	14 (4.1%)
1514	<i>Strongyloides stercoralis</i> (rhabditiform larvae)	327	284 (86.9%)		14 (4.3%)	7 (2.1%)	16 (4.9%)	6 (1.8%)
2255	<i>Strongyloides stercoralis</i> †† (filariform larvae)	321	127 (39.6%)		116 (36.1%)	38 (11.8%)	40 (12.5%)	
1600	<i>Enterobius vermicularis</i>	339	299 (88.2%)			5 (1.5%)	35 (10.3%)	
Cestodes:								
1335	<i>Hymenolepis nana</i>	314	192 (61.1%)	14 (4.5%)	4 (1.3%)	18 (5.7%)	32 (10.2%)	54 (17.2%)
2369	<i>Hymenolepis nana</i>	343	258 (75.3%)	7 (2.0%)	16 (4.7%)	7 (2.0%)	24 (7.0%)	31 (9.0%)
1974	<i>Hymenolepis diminuta</i>	342	259 (75.7%)	13 (3.8%)	22 (6.4%)	43 (12.6%)	5 (1.5%)	
1474	<i>Taenia</i> sp	326	299 (91.7%)		1 (0.3%)	11 (3.4%)	2 (0.6%)	13 (4.0%)
1654	<i>Taenia</i> sp	345	310 (89.8%)	2 (0.6%)	7 (2.0%)	13 (3.8%)	2 (0.6%)	11 (3.2%)
1941	<i>Taenia</i> sp	324	284 (87.8%)		3 (0.9%)	27 (8.3%)	5 (1.5%)	5 (1.5%)
1573	<i>Echinococcus granulosus</i> #	339	234 (69.1%)		33 (9.7%)	14 (4.1%)	57 (16.8%)	1 (0.3%)
1916	<i>Echinococcus granulosus</i> #	322	301 (93.5%)		3 (0.9%)	5 (1.6%)	12 (3.7%)	1 (0.3%)
2200	<i>Echinococcus granulosus</i> #	351	338 (96.3%)		6 (1.7%)	4 (1.1%)	3 (0.9%)	
2406	<i>Echinococcus granulosus</i> #	339	312 (92.0%)		18 (5.3%)	6 (1.8%)	2 (0.6%)	1 (0.3%)
Trematodes:								
1433	<i>Fasciola hepatica</i>	331	177** (53.3%)			17 (5.1%)	130 (39.3%)	7 (2.1%)
1973	<i>Fasciola hepatica</i>	329	290** (88.2%)		4 (1.2%)	22 (6.7%)	7 (2.1%)	6 (1.8%)
1546	<i>Clonorchis sinensis</i>	340	325** (95.6%)		1 (0.3%)	9 (2.6%)	3 (0.9%)	2 (0.6%)
1874	<i>Clonorchis sinensis</i>	344	335** (97.4%)			4 (1.2%)		5 (1.4%)
1684	<i>Schistosoma mansoni</i>	343	309 (90.1%)				34 (9.9%)	
1845	<i>Schistosoma mansoni</i>	342	307 (89.8%)	1 (0.3%)	1 (0.3%)	4 (1.2%)	22 (6.4%)	7 (2.0%)
2284	<i>Schistosoma mansoni</i>	354	297 (83.9%)	1 (0.3%)	4 (1.1%)	6 (1.7%)	27 (7.6%)	19 (5.4%)
1353	<i>Schistosoma haematobium</i> ###	325	232 (71.4%)				92 (28.3%)	1 (0.3%)
1846	<i>Schistosoma haematobium</i> ###	342	303 (88.6%)	4 (1.2%)			34 (9.9%)	1 (0.3%)
2253	<i>Schistosoma haematobium</i> ###	354	349 (98.6%)		3 (0.8%)		2 (0.6%)	
1975	<i>Schistosoma mekongi</i>	342	280 (81.9%)	7 (2.0%)	2 (0.6%)	24 (7.0%)	13 (3.8%)	16 (4.7%)
Protozoa:								
1351	<i>Entamoeba coli</i>	326	229 (70.3%)		3 (0.9%)		3 (0.9%)	91 (27.9%)
1382	<i>Entamoeba histolytica</i>	337	277 (82.2%)	6 (1.8%)	8 (2.4%)	5 (1.5%)		41 (12.1%)
1915	<i>Entamoeba histolytica</i>	321	272 (84.7%)	3 (0.9%)		8 (2.5%)	4 (1.3%)	34 (10.6%)
1475	<i>Iodamoeba buschlii</i>	326	279 (85.6%)		2 (0.6%)	8 (2.5%)	7 (2.1%)	30 (9.2%)
1574	<i>Chilomastix mesnili</i>	340	235 (69.1%)			73 (21.5%)	19 (5.6%)	13 (3.8%)
2321	<i>Chilomastix mesnili</i>	336	242 (72.0%)			47 (14.0%)	30 (8.9%)	17 (5.1%)
1404	<i>Endolimax nana</i>	323	189 (58.5%)		17 (5.3%)	39 (12.1%)	57 (17.6%)	21 (6.5%)
1547	<i>Giardia lamblia</i> (cysts)	340	333 (97.9%)			1 (0.3%)	5 (1.5%)	1 (0.3%)
1954	<i>Giardia lamblia</i> (cysts)	330	308 (93.3%)			3 (0.9%)	19 (5.8%)	
1972	<i>Giardia lamblia</i> (cysts)	329	313 (95.2%)			6 (1.8%)	6 (1.8%)	4 (1.2%)
1711	<i>Giardia lamblia</i> (trophozoites)	343	300 (87.5%)			5 (1.5%)	38 (11.0%)	
Coccidia:								
1434	<i>Cryptosporidium</i> sp	318	286 (89.9%)				32 (10.1%)	
1761	<i>Cryptosporidium</i> sp	340	309 (90.9%)				31 (9.1%)	
1875	<i>Cryptosporidium</i> sp	340	309 (90.9%)				31 (9.1%)	
2176	<i>Cryptosporidium</i> sp	348	332 (95.4%)			1 (0.3%)	15 (4.3%)	
2362	<i>Cryptosporidium</i> sp	336	321 (95.5%)				15 (4.5%)	

*Some non-faecal parasites included. **Includes visually indistinguishable ova. †Most reports stated *Strongyloides stercoralis* (misidentification). ††Sputum sample containing filariform larvae of *Strongyloides stercoralis*. #Hydatid cyst fluid samples. ##Terminal urine samples. ###From specimen 2150 onwards, incorrect reporting of stages present was penalised in scoring.

Table 4 Mixed faecal specimens distributed and participants' results

Specimen numbers	Species	Number of laboratories	Three parasites found*	Two parasites found*	One parasite found*	No parasites found	Unexpected parasites only
1403	Hookworm sp and <i>Trichuris trichiura</i>	323	N/A	177 (54.8%)	135 (41.8%)	10 (3.1%)	1 (0.3%)
1627	<i>Endolimax nana</i> and <i>Entamoeba coli</i>	340	N/A	181 (53.2%)	125 (36.8%)	16 (4.7%)	18 (5.3%)
1653	<i>Entamoeba histolytica</i> and <i>Entamoeba coli</i>	344	N/A	276 (80.3%)	61 (17.7%)		7 (2.0%)
2322	<i>Entamoeba histolytica</i> and <i>Entamoeba coli</i>	336	N/A	97** (28.9%)	203 (60.4%)	26 (7.7%)	10 (3.0%)
1762	<i>Chilomastix mesnili</i> and <i>Entamoeba coli</i>	323	N/A	135 (41.8%)	183 (56.7%)	2 (0.6%)	3 (0.9%)
2150	<i>Entamoeba histolytica</i> , <i>Entamoeba coli</i> , and <i>Giardia lamblia</i>	349	28 (8.0%)	201 (57.6%)	106 (30.4%)	9 (2.6%)	5 (1.4%)
2175	<i>Hymenolepis nana</i> , <i>Entamoeba coli</i> and rhabditiform larvae of <i>Strongyloides stercoralis</i>	360	210 (58.3%)	102 (28.3%)	44 (12.2%)	2 (0.6%)	2 (0.6%)
2198	<i>Ascaris lumbricoides</i> and Hookworm sp	354	N/A	322 (91.0%)	32 (9.0%)		
2254	<i>Taenia</i> sp and <i>Endolimax nana</i>	354	N/A	155 (43.8%)	167 (47.2%)	19 (5.3%)	13 (3.7%)

N/A: Not applicable.

*May include the presence of unexpected parasites or obvious misidentification of parasites present.

**This specimen contained very low cyst numbers.

Table 5 Distribution of cumulative scores for blood/tissue and faecal specimens

Per cent of total possible score	100-81	80-61	60-41	40-21	< 20
Faecal parasitology:					
No of achieving laboratories	60	131	55	13	4
Cumulative per cent of laboratories achieving	(22.8%)	(49.8%)	(20.9%)	(4.9%)	(1.5%)
Blood parasitology:					
No of achieving laboratories	35	56	19	8	2
Cumulative per cent of laboratories achieving	(29.2%)	(46.6%)	(15.8%)	(6.7%)	(1.7%)

Cumulative scores are shown for the 120 laboratories that returned reports on all seven blood/tissue specimens (total possible score of 16) and the 263 laboratories that returned reports on all 12 faecal specimens (total possible score of 40) distributed from June 1990-June 1991.

coal culture,⁶ before adding to prescreened faeces containing either no parasites or additional known parasites. *Echinococcus granulosus* was obtained from cyst fluid and *Fasciola hepatica* and *Cryptosporidium* spp from veterinary sources. Latex beads were purchased from Coulter Electronics Ltd (Northwell Drive, Luton, Bedfordshire, England). The amastigotes of *Leishmania* spp were obtained from impression smears of hamster spleen and the *Trypanosoma* spp from the blood of infected rats. Faecal samples were homogenised until semi-liquid using an industrial blender, parasite numbers were assessed using the modified formol-ether method⁶ and faecal suspensions for distribution were diluted with 10% v/v formol-water (1 in 10 dilution of 37-41% w/v formaldehyde) as necessary to adjust the concentration of parasites. Specimens distributed as concentrates were produced from pooled concentrates of a single specimen using the modified formol-ether method. Faecal samples were emulsified in 10% v/v formol-water, filtered to remove coarse material; faecal fats were extracted by vortexing the filtrate in diethyl ether and then parasites were sedimented by centrifugation. Faecal concentrates and faecal suspensions were aliquoted by peristaltic dispenser into 2 ml phials which were then sealed with a rubber stopper and an aluminium cap.

Thin blood smears were fixed in methanol for one minute and stained with Giemsa stain (1 in 20 dilution for one hour at pH 6.8 or pH

7.2 depending on the parasite present). Slides of tissue parasites were distributed as unstained impression smears or as tissue fluid dried on to slides and fixed in methanol.

The quality of the samples and specimens was checked before and after bottling. For faecal samples, concentrations were made throughout the preparation process and following final dilution of the sample. Ten (2%) phials were selected at random from about 500 phials bottled and the numbers of all parasites present in the samples were counted. Five (2%) samples selected randomly from the 250 slides made from a blood specimen were examined for adequate staining and parasite morphology. Five samples of each faecal specimen and two samples of each blood specimen were returned by selected participants to HTD where they were examined to exclude deterioration of the specimen between preparation and receipt by the participants.

Specimens were labelled and packed in accordance with United Kingdom, and where appropriate, international regulations for post-age of pathological specimens.

DOCUMENTS

Documents accompanied each specimen informing participants of the type of specimen, the examination required, any hazards associated with the specimen and clinical details leading to the request for examination. A questionnaire requesting details of methods used was distributed on one occasion. United Kingdom participants were asked to return reports of their results to the HTD within three weeks of distribution and overseas participants within four weeks. Following assessment and scoring of results at HTD, a summary of performance was sent to participants together with an individual computer printout showing details of performance both with current specimens and those examined over the previous 12 months, the mean of all participants' scores, and the standard error of the mean. Performance during the year was summarised for each laboratory in an annual report and individual printout of results. Educational text was dis-

tributed on numerous occasions where an unusual parasite was distributed or where problems in performance were noted.

SCORING SCHEME

A score was allocated for each result returned by participants. Until late 1989, a four point scoring scheme based on that used in most of the other microbiology quality assessment schemes was used.⁷ A score of 2 was awarded for fully correct results, 1 for partially correct results, - 1 where unexpected pathogens were reported and 0 for unexpected non-pathogens, no parasites found, or inadequate identification of parasites. In 1990 a more discriminatory scoring scheme was introduced, based on the addition or subtraction of points for three components of participants' reports; recognition of the presence of a parasite; the level and correctness of identification and absence of unexpected parasites. With positive specimens, two points were added for each parasite present fully and correctly identified and one point for each parasite present incompletely characterised—that is, to genus level only or the wrong stage described. Zero points were given for incorrect speciation of parasites present or for each parasite present and not found or inadequately characterised. One point was subtracted where the incorrect stage of a parasite was described in addition to the correct stage and two points were subtracted where incorrect species were described in addition to correct species and for each unexpected parasite found (up to a maximum of three). With negative specimens, two points were awarded for a correct report of no parasites found and two points was subtracted for each unexpected parasite found (up to a maximum of three). The range of scores attainable for each specimen thus depended on the number of parasites included, varying between - 6 to 2 for a negative specimen or one containing a single parasite, between - 6 to 4 for a specimen containing two parasites and between - 6 to 6 for a specimen containing three parasites.

Results

BLOOD PARASITES

Results of United Kingdom participants with blood specimens are shown in tables 1 and 2. As an illustrative example for a 12 month period, the distribution of cumulative scores achieved by laboratories examining all seven specimens distributed from June 1990–June 1991 is shown in table 5. The average cumulative score for the series was 69.1%. Some 29.2% of laboratories achieved 81% or greater of their total possible scores.

FAECAL PARASITES

Results of United Kingdom participants with faecal specimens are shown in table 3 (single parasites) and table 4 (mixed parasites). Specimens 1736 and 2342 contained latex beads with a mean measurement of 9.4 μm , distributed as measurement exercises. With specimens 1736, 113 of 273 laboratories (41.4%)

reported measurement within the range 9.1–10.0 μm (+/- 2SD from mean); with specimen 2342, 138 of 314 laboratories (43.9%) reported measurement within the range 9.1–10.0 μm (+/- 2SD from mean). Six negative faecal specimens were distributed, with unexpected parasites reported by between 2.6% and 17.9% of participants. A wide range of unexpected parasites were reported, including most parasites found in human intestinal infections.

The distribution of cumulative scores achieved by laboratories examining all 12 specimens distributed from June 1990–June 1991 is shown in table 5. The average cumulative score for the series was 68.3%. Some 22.8% of laboratories achieved 81% or greater of their total possible scores.

QUESTIONNAIRE

A questionnaire requesting details of methods used was distributed in August 1990 to 463 participants and completed questionnaires were received from 314. Of the 291 laboratories specifying methods used for faecal concentration, 216 used formol-ether concentration, 57 used the "Evergreen" commercial kit (Evergreen Scientific, USA), five used the "Parakit" commercial kit (Meridian, Launch Diagnostics), and four used ZnSO₄ flotation. Various other methods were used by nine participants.

Of the 226 participants using one or more stains in the examination of faecal preparations and smears, 206 used iodine, 35 Sargeant's, 12 eosin, 10 trichrome, eight Burrow's, five acridine, five iron haematoxylin, four Giemsa and two Field's. Various other methods were used by single participants.

One or more stains for the identification of *Cryptosporidium* sp were used by 199 participants of which 133 used modified Ziehl Neelsen, 85 auramine, 10 safranin/methylene blue and three MWE "Rapid Crypto" (Medical Wire Equipment).

Of the 135 participants using one or more stains for blood films, 105 used Giemsa, 17 Leishman, 12 May-Grünwald Giemsa, 12 Field's and 10 modified Wright's. Various other methods were used by 11 participants.

Eye-piece graticules were used for measurement with faecal specimens by 246 participants, were not used by 65; three did not answer this question.

Discussion

The organisation of a quality assessment scheme for parasitology is difficult, especially for faecal parasitology. Relatively few clinically important parasites can be grown in artificial culture or propagated in laboratory animals and clinical material is the main source of supply. Few centres in developed countries see a sufficient diversity of infections to provide material for a comprehensive quality assessment scheme. It is also difficult to collect adequate volumes of faecal material for each specimen, 1 g of faeces being required for each participant plus additional material for ade-

quate quality control and for providing repeat specimens to participants failing with the first. Such quantities of material are easiest to collect from inpatients with the active collaboration of ward staff. Increasingly parasitic infections are treated in the outpatient department, which aggravates the problem of supply, as does the growth of the scheme, both in the numbers of participants and in associated teaching activities. For these reasons, it has been found necessary to undertake periodic visits to the tropics to collect material for use in the scheme.

Other problems are inherent in the use of biological material. It is difficult to ensure consistency of numbers of parasites between phials, particularly where multiple parasites or low numbers are included. Semi-liquification of the sample, thorough blending, and mixing during bottling reduce sample variation. Shortage of clinical material precludes the inclusion of "text book" examples of parasites on every occasion, and some degeneration during transport may further alter morphological features, although examination by the HTD of duplicate samples returned through the post controlled this possibility. Judgement is required to ensure that in spite of these factors the morphological features of parasites distributed are representative of those encountered in the often less than ideal specimens in the clinical laboratory. As well as potentially causing heterogeneity within a single specimen, variation among specimens can also complicate comparison of participants' results with parasites distributed repeatedly. To overcome this problem, where sufficient material is available and where parasites are stable on storage, a sample is divided into quantities sufficient for two or more distributions. The absence of very low numbers of parasites other than those seen in the organising laboratory is impossible to guarantee because there is a limit to the volume of sample that can be screened. However, participants' reporting of unexpected parasites in terms of numbers and variety was far greater than could be attributed to inadequate pre-distribution screening. Performance with negative specimens has shown some variation (table 1) in the numbers of participants reporting the presence of unexpected parasites, probably due to differences in the content of undigested elements (such as fruit fibres, cellulose, and meat fibres), leading to misidentification as ova and larvae. The reporting of unexpected parasites in positive samples containing parasites may be caused by misidentifications of artefacts or of the parasites present (table 3). Several of the specimens listed in table 3 show high numbers of participants reporting the presence of unexpected parasites—for example, specimen 1404 (12.1%), specimen 1574 (21.5%), specimen 1737 (38.0%), specimen 1974 (12.6%) and specimen 2255 (11.8%). In most cases this is due to confusion of the actual species present, whether ova or cysts, with another species of similar appearance and size. Although participants were invited to submit photomicrographs in support of such findings, this was

rarely done.

Scoring of participants' results is useful in assessing overall standards of performance with time, and in providing participants with a measure of their individual performance compared with their peers. This has proved more difficult than in other areas of microbiology because of the complexity of possible combinations of response in terms of numbers of actual parasites present, unexpected parasites reported, and the level of identification made. It is rare to find single parasites in clinical specimens and to reflect this, quality assurance specimens contained up to three parasites. Identification of most parasites relies mainly on correct morphological identification and speciation is necessary to confirm or exclude pathogenicity. For this reason, unlike other microbiology subschemes, speciation of non-pathogens is required to obtain a full score. The four point scoring scheme used for most other microbiology subschemes lacked adequate discrimination; the scheme adopted for parasitology allowed more logical scoring and provided a more objective measure of performance. It is, however, more confusing for participants since the maximum score attainable is not constant, varying between 2 and 6, depending on the numbers of parasites present in the specimen. The scoring scheme at present lacks a penalty for incorrect answers which could lead to clinical catastrophe, as for example, misdiagnosis of a *Plasmodium falciparum* infection.

The overall standard of performance with these quality assessment specimens varied. At best, success rates of between 90–99 % of participants achieving full scores with some specimens comparing favourably with many of the other microbiology subschemes. However, there are considerable variations in success rates where parasites have been repeatedly distributed on separate occasions (tables 1 to 4). These differences may reflect variations between specimens such as the number of species of parasites present, the variable quantity of each species present, and the state of degeneration. Comparison between specimens is further complicated by changes in the scoring scheme introduced in June 1990 and a concurrent change from faecal concentrates to faecal suspensions.

A noticeable drop in performance was noted with repeated distribution of a mixture containing cysts of *Entamoeba coli* and *Entamoeba histolytica* (table 4). The initial sample (1653) was distributed as a faecal concentrate containing moderate numbers of cysts from both species and 80.3% of participants reported a fully correct result. The second sample (2322) was distributed as a faecal suspension for a concentration containing very low numbers of cysts, with only 28.9% of participants reporting a fully correct result. This much poorer performance could possibly be attributed to inadequate concentration of the sample or a failure fully to examine the entire sample.

Repeated distribution of less commonly encountered species has resulted in an encouraging trend towards improved performance.

For example, the percentages of laboratories achieving fully correct results in successive distributions were, for *Hymenolepis nana*, 61.1% and 75.3%; for *Fasciola hepatica*, 53.3% and 88.2%; and for *Schistosoma haematobium*, 71.4%, 88.6%, and 98.6% (table 3).

A sputum sample containing filariform larvae of *Strongyloides stercoralis* was distributed as specimen 2255 (table 3). Fully correct results were achieved by 39.6% of participants. The sample also contained some yeasts which were misidentified as *Pneumocystis carinii* by 29 of 38 (78.3%) of laboratories reporting unexpected parasites. Extensive testing of the sputum sample prior to distribution showed no evidence of *P. carinii*.

Poor performance of detection of malarial parasites is mainly attributable to incorrect speciation, description of the wrong stages of parasites, and diagnosis of a mixed *Plasmodium* infection when only one species is present. Incorrect speciation occurs through lack of familiarity with species differences such as infected cell size and shape, parasite appearance within the cell, pigment characteristics, presence or absence of Maurer's clefts or Schuffner's dots and poor knowledge of the geographical distribution of species. Unexpected parasites reported in a sample containing malarial parasites are often the result of misidentification of artefacts and staining deposit or identifying exflagellating gametocytes as *Trypanosoma* sp or *Borrelia* sp. The error in estimation of percentage parasitaemias of *P. falciparum* infections appears greatest when high numbers of parasites are present, with the poorest performance noted with specimen 2323 (23% parasitaemia) (table 2). This could be due to laboratories using inappropriate methods of estimation such as counting more than one parasite per infected cell.

Microfilariae are poorly speciated by participants. Clinical details accompanying specimens aim to aid participants in their final diagnosis of the species present. In most cases a misidentification within the group of microfilariae cannot be considered an incorrect speciation as most genera of microfilariae contain only one species. Unexpected parasites reported with microfilariae samples (table 1) usually represent misidentification of the microfilariae present, rather than the reporting of unrelated unexpected parasites, such as specimen 2151, where 27.1% (36 of 133) of participants reported unexpected parasites and other microfilariae accounted for 88.9% (32/36) of these reports.

Distributions of latex beads for measurement showed that as many as 20% of participants receiving faecal distributions did not possess an eyepiece graticule and were unable to perform measurements. This is a serious concern as measurement is essential for accurate diagnosis and differentiation of parasite ova and cysts. The wide range of measurements reported indicated that graticules in use by some participants may have been incorrectly calibrated. Results from the questionnaire showed a variation in methods used for faecal concentration. The method recommen-

ded by the Department of Clinical Parasitology at HTD is modified formol-ether concentration.⁸ The use of gauze for the filtration of coarse material from faecal samples is inappropriate as parasites, especially ova, can be retained on its surface and wire mesh should be used. The samples of faecal suspension were intended for concentration, using the entire sample provided. All statistical data provided by QAL are based on the average numbers of parasites present in one phial of sample. Those participants who did not follow the instructions and divided a sample into several aliquots or treated the specimen as a pre-prepared faecal concentrate performed poorly as a result. Laboratories achieving incorrect results are encouraged to obtain repeat samples to investigate problems. Further assistance regarding technical matters is available from the Department of Clinical Parasitology, HTD. A mechanism for offering advice and help to poorly performing laboratories through the Advisory Panel for Microbiology² was introduced in July 1991.

Since the closure of the IMLS fellowship course in 1972, there have been fewer opportunities for specialised training in parasitology. This problem has been addressed in part by short courses in blood and intestinal parasitology held at Paddington College (Polytechnic of Central London), but these courses are over-subscribed and accessible to only a small number of participants. Other short courses are also available at the London and Liverpool Schools of Tropical Medicine and a part time MSc course in clinical parasitology is now offered by the Royal Free Hospital School of Medicine together with the London School of Hygiene and Tropical Medicine. There are currently plans to introduce, as part of the quality assessment scheme, an expanded teaching programme, offering greater accessibility to teaching for all participants. It is hoped to achieve this by running seminars in blood and intestinal parasitology at regular intervals in various locations throughout the United Kingdom.

The parasitology schemes aim to offer an interesting and challenging programme of assessment and teaching, and it is hoped that laboratories' knowledge, confidence, and interest in parasitology will continue to grow.

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