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Accuracy of routine laboratory diagnosis of malaria in the United Kingdom

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Abstract

***Aims*—To study the accuracy of routine laboratory diagnosis of malaria with the aim of improving accuracy in diagnosis in the future.**

***Methods*—A comparative study was made of all blood films submitted to two laboratories in London providing a slide-diagnostic service for malaria.**

***Results*—There were 17 *Plasmodium ovale* infections, and of these only five (29·4%) were correctly diagnosed by the submitting laboratory; whereas of 210 other single species infections, 162 (77·1%) were correctly diagnosed (χ^2 = 18·4, p < 0·0001). There were six patients with mixed infections; only one (16·7%) was correctly diagnosed, whereas of 227 single species infections, 167 (73·6%) were correctly diagnosed (p = 0·007, using Fisher's exact test). There was no significant association between the presence of technical faults or numerous platelets and incorrect diagnosis.**

***Conclusions*—*Plasmodium ovale* and mixed infections were diagnosed incorrectly significantly more often than other species. The presence of technical faults or numerous platelets had no significant effect on whether or not submitting laboratories correctly diagnosed malaria.**

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In 1991 there were 2332 cases of malaria reported in the United Kingdom with 12 deaths, 11 due to *Plasmodium falciparum*.[1]

The laboratory diagnosis of malaria is based on identifying parasites in peripheral blood by examination of thick and thin blood films.[2–5] Most slides made in the United Kingdom where malaria is considered a possible clinical diagnosis are submitted for verification to the Public Health Laboratory Service Malaria Reference Laboratory (MRL), or the Hospital for Tropical Diseases (HTD). In addition, HTD organises the United Kingdom National External Quality Assessment Scheme (UK NEQAS) for blood parasitology.

We have undertaken a comparative study of all blood films submitted to these two reference laboratories for confirmation or exclusion of malaria parasites. We attempted to find out the accuracy of routine laboratory diagnosis of malaria in the United Kingdom with the aim of improving accuracy in diagnosis in the future.

Methods

The study was conducted from 9 March to 31 May 1992 at the MRL and HTD. All blood films submitted during those dates for confirmation or exclusion of malaria were examined. Ninety one laboratories in the United Kingdom submitted blood films from a total of 267 patients. There were thick and thin blood films from 104 patients, thin films only from 161, and thick films only from two. A specimen was defined as the blood film/s received from one patient. The type of referring department was recorded. The malaria diagnosis made at the reference laboratories was defined as the true diagnosis and this was compared with the diagnosis made by the submitting laboratory as stated on the request form. Their diagnosis was regarded as correct if it stated the same malaria species as was reported by the reference laboratories, but incorrect if it did not, or if malaria parasites had been reported to be present by the submitting laboratory, but not seen by the reference laboratories.

Films which had been stained for the presence of malaria parasites before submission and those stained by the reference laboratories using standard methods[3] were technically assessed. It was assumed that slides stained by the reference laboratories were stained correctly. The presence or absence of technical faults based on WHO criteria[4] were recorded for pH of stain used (acidic, alkaline, appropriate), stain debris (present, absent), film spread on greasy slide (yes, no), fixation (poor, adequate), depth of thick film (too thin, too thick, appropriate), edge of spreader slide chipped (yes/no). Sequestrene effects (yes, no), which were defined as films containing crenated red blood cells or neutrophils showing vacuolation, swelling of the lobes of the nuclei, or disintegration[5] were noted. The occurrence of individual instances or combinations of these faults was recorded. Thin blood films containing numerous platelets (more than five per field when examined microscopically at ×1000 magnification) were also recorded.

Statistical analysis was done using Epi Info (Centers for Disease Control, Atlanta, Georgia). Tables were drawn up to test the null hypothesis that incorrect diagnoses from submitting laboratories were unrelated to the type of department submitting the specimen, the species of malaria diagnosed by the reference laboratories, or the presence of technical faults or films containing numerous platelets.

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Table 1 Comparison of diagnoses made by receiving laboratories (true diagnosis) and submitting laboratories

Receiving laboratories	*Patients*	*Submitting laboratories*	*(% of patients)*
P falciparum	112	*P falciparum*	85 (75·9%)
		? *P falciparum*	3 (2·7%)
		Incomplete	18 (16·1%)
		Other*	6 (5·3%)
P vivax	94	*P vivax*	59 (62·8%)
		? *P vivax*	13 (13·8%)
		Incomplete	16 (17%)
		Other†	6 (6·4%)
P ovale	17	*P ovale*	5 (29·4%)
		Incomplete	6 (35·3%)
		Other†	6 (35·3%)
P malariae	4	*P malariae*	1 (25%)
		? *P malariae*	1 (25%)
		Other§	2 (50%)
Mixed infections	6	Please see table 2	
No malaria parasites seen	34	No malaria parasites seen	21 (61·8%)
		Incomplete	2 (5·8%)
		Other‖	11 (32·4%)

Other* *P vivax* 3, ? *P vivax* 1, *P malariae* 1, ? *P falciparum*/? *P vivax* 1
Other† ? *P falciparum* 1, *P vivax and P falciparum* 1, ? *P vivax*/? mixed infection 1, ? *P vivax*/? *P ovale* 1, ? *P malariae* 1, ? *P ovale*/? *P malariae* 1
Other‡ ? *P falciparum* 1, *P vivax* 4, *P malariae* 1
Other§ *P vivax* 1, No details on form 1
Other‖ *P falciparum* 2, *P vivax* 3, *P ovale* 1, ? *P malariae* 1, ? Malaria parasites present 4

The results were tested using the χ^2 test or, if appropriate, Fisher's exact test.

Results

Blood films were submitted from various departments: from haematology (n = 79), microbiology (n = 8), pathology (n = 2), one each from immunology and obstetrics. There was no significant association between the source submitting the blood films (haematology department or other) and incorrect diagnosis. The numbers of patients' blood films referred by individual laboratories varied from one (44 laboratories) to 21 (one laboratory).

Table 1 shows the diagnosis of the reference compared with the submitting laboratories. According to our assessment, blood films from 227 out of 267 patients contained one species of malaria, six patients had mixed infections with two species, and in films from 34 patients malaria parasites were not seen. Incomplete results apply to those submitting laboratories which identified malaria parasites but did not state the species. There were 17 *P ovale* infections, and of these, only five (29·4%) were correctly diagnosed, whereas of the 210 remaining single infections, 162 (77·1%) were correctly diagnosed ($\chi^2 = 18{\cdot}4$, $p < 0{\cdot}0001$).

Table 2 shows that of six mixed infections, only one (16·7%) was correctly diagnosed by the submitting laboratories, whereas of the 227 single strain infections, 167 (73·6%) were correctly diagnosed ($p = 0{\cdot}007$ using Fisher's exact test). In three infections *P falciparum* was identified but the second species present was missed.

There were 104 individual technical faults from 82 specimens. These were: acidic pH used in 33 specimens; acidic pH used and stain debris present in eight; acidic pH used and greasy slide in one; acidic pH used and inadequate fixation in five; acidic pH used, inadequate fixation, and stain debris present in two; acidic pH used and thick films too thin in one; stain debris present in 12; spreader slide chipped in one; stain debris present and greasy slide in one; greasy slide used in three; greasy slide and inadequate fixation in two; thick film too thin in four; and inadequate fixation in nine. Sequestrene effects were also present in 216 of 254 specimens examined, and in 150 of these the correct diagnosis was given by the submitting laboratory. Numerous platelets were present in 15 specimens. In 12 of these the correct diagnosis was given by the submitting laboratory, and the remaining three were incorrectly identified. One was thought to be *P falciparum* by the submitting laboratory; the other two were incompletely identified. There was no significant association between the presence of the various technical faults or numerous platelets and incorrect diagnoses made by the submitting laboratories.

Table 2 Comparison of diagnoses made in patients with mixed infections

Receiving laboratory result	*Submitting laboratory result*
P falciparum and *P vivax*	*P falciparum* and *P vivax*
P falciparum and *P vivax*	*P falciparum*
P falciparum and *P vivax*	Incomplete
P falciparum and *P ovale*	*P falciparum*
P falciparum and *P ovale*	*P falciparum* and ? *P malariae*
P falciparum and *P malariae*	*P falciparum*

Discussion

During this study blood films from 243 patients with malaria in England and Wales were submitted to our laboratories, compared with 183 cases of malaria recorded by the Office of Population Censuses and Surveys from 7 March to week ending 29 May 1992.[6,7] Although the number of patients common to both groups is unknown, we think that most slides from patients with malaria are being submitted to the MRL or HTD. *P falciparum* was also correctly identified in 78·6% of our cases compared with an average of 74·7% in the United Kingdom National External Quality Assessment Scheme,[8] where optimally stained material is sent mainly to microbiologists. The corresponding figures for *P vivax* were 76·6% and 71·4%, respectively. This is helpful confirmation as the time span of our study was less than three months, but the NEQAS Parasitology Subscheme has been in existence since 1986. Thus our submitting laboratories were similar in performance to those participating in the NEQAS, and represent the average diagnostic laboratory. We conclude that our study represents the general situation in the United Kingdom.

As the microscopical identification of parasites in blood is the most certain method of confirming infection with malaria parasites,[5,9] it is to be expected that haematology departments would undertake most malaria investigations as was the case in this study. However, other departments such as microbiology may occasionally be involved and the performance of other types of department did not differ significantly in this study.

Table 1 shows that mistakes were made in the diagnosis of all species of malaria. Five of 112 patients with *P falciparum* were regarded by the submitting laboratories as being infected with another species of malaria. Patients with this infection may deteriorate rapidly and it constitutes a medical emergency. Current

treatment for *P falciparum* differs from that of the other species infecting people. The case fatality rate due to *P falciparum* is of the order of 1% in the United Kingdom[10] and delay in diagnosis is a recognised factor contributing to a fatal outcome. In the case of *P vivax* mortality in the United Kingdom due to this infection is negligible,[10] but mistakes in laboratory diagnosis are still important, particularly if *P falciparum* is diagnosed by the submitting laboratories, as primaquine, which is given to eliminate hypnozoites of *P vivax* from the liver, is not required in *P falciparum* infections. *P ovale* was correctly diagnosed significantly less often than the other species ($\chi^2 = 18{\cdot}4$; $p < 0{\cdot}0001$). For *P ovale*, four infections were reported as *P vivax* by the submitting laboratories. These two species are similar in appearance,[9] and this may be the reason for the confusion. Fortunately, the treatment regimens would be identical. In 13 of 34 cases where malaria parasites were not seen by the reference laboratory malaria parasites were reported to be present or possibly present by the submitting laboratories. All species of malaria were reported, and a common error is the detection of malaria parasites where none is present.[2]

Table 2 shows that only one of six (16·7%) mixed infections was correctly identified by the submitting laboratory. This was significantly less than for single strain infections ($p = 0{\cdot}007$, using Fisher's exact test). However, *P falciparum* was correctly identified in five of six mixed infections. There is a need for greater awareness by submitting laboratories of the possibility of infection with more than one species of malaria simultaneously, and the number of mixed species infections reported in the United Kingdom doubled between 1986 and 1989–90.[11]

Technical faults were sought, based on those identified by the WHO,[4] and their effect on the diagnosis made by the submitting laboratory was examined. When examination for malaria parasites is undertaken, thin films are initially fixed in methanol before being stained.[3] For 18 specimens this procedure had been performed poorly because their erythrocytes floated on the slide while we undertook microscopical examination. A common fault was films stained at an acidic pH—that is, pH 6·8–7 according to WHO[4]—which occurred in 50 specimens. The detection of malaria parasites in blood involves the use of differential stains which stain the nuclear material red and cytoplasm blue[3 5] with clearly visible red blood cell stippling.[3 5] These appearances will only occur if the pH used during staining is pH 7·2,[3] as previous authors have emphasised.[2 5] For example, Schuffner's dots due to *P vivax* will not be seen if pH 6·8 is used. Our results suggest that although routine haematology laboratories usually stain blood films at pH 6·8,[2] some are inappropriately examining for the presence of malaria parasites at this pH. Stain debris was present on the blood films of 23 specimens. These artefacts may cause confusion in diagnosis[4] and may be identified as malaria parasites.[2] Sequestrene effects were seen in 85% of specimens examined, which is to be expected as they occur within two to three hours of storage in EDTA.[5] Despite the presence of various technical faults, they did not occur more commonly when submitting laboratories gave incorrect rather than correct diagnoses. Slides containing numerous platelets were not reported incorrectly more often than those without this feature. It is known that platelets, if superimposed on erythrocytes, may cause confusion in the diagnosis of malaria,[2] and if several platelets are superimposed they may be mistaken for malaria parasites outside the red blood cell.[9]

Our study has shown that there is a need to improve the accuracy of routine laboratory diagnosis of malaria. This could be achieved if routine laboratories continue to gain experience by participating in external quality assurance schemes such as NEQAS. We have shown that their internal standard operating procedures may produce results which are technically faulty, as defined by WHO criteria.[4] Although technical faults had no significant effect on the accuracy of diagnosis in our study, others[2 4] have reported an effect. Laboratories may wish to discuss aspects of their standard operating procedures with designated experts, and must have good communications with their reference laboratories.

In conclusion, this study found that there were inaccuracies in the diagnosis of all species of malaria especially for *P ovale*. The most serious inaccuracy was that five of 112 patients infected with *P falciparum* were thought to be infected with another species. Only one of six mixed infections was accurately diagnosed and there was a lack of awareness of this diagnostic possibility by the submitting laboratories. The presence of various technical faults or numerous platelets did not differ significantly among submitting laboratories reporting correct or incorrect diagnoses.

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