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Detection and Identification of Malaria Parasites: A Review of Proficiency Test Results and Laboratory Practices

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Abstract

Background: This study assesses proficiency test (PT) performance in detecting and identifying malaria parasites and examines malaria screening practices in laboratories in the United States.

Method: We reviewed PT data from 1999 to 2008 to determine the rates of unacceptable responses for detecting and identifying *Plasmodium* sp. We also surveyed current PT participants to assess malaria screening practices.

Results: Laboratories were most proficient at detecting and identifying *P falciparum* and substantially less proficient at identifying *P malariae*, *P vivax*, and *P ovale*. Half of the surveyed laboratories failed to offer a complete diagnostic workup, 25.0% failed to screen both thick and thin slides, and 36.1% failed to issue a final report within 24 hours.

Conclusion: Educational programs should focus on identifying *Plasmodium* sp. and distinguishing artifacts from parasites. Laboratories should offer a complete diagnostic workup, either in-house or by referral, and ensure final results within 24 hours.

Keywords: malaria, *Plasmodium*, proficiency testing, microbiology

Malaria, which is caused by *Plasmodium* parasites, was common in the southeastern United States during the first half of the last century. As a result of aggressive mosquito control and environmental efforts, the incidence of malaria in this country declined from about 600,000 cases in 1914 to only 1505 cases in 2007, and transmission of the disease by mosquitoes is now rare.[1] Instead, most cases of malaria in the United States now occur in persons who have traveled to an area where malaria is endemic. Less often, it is transmitted by transfusions of blood or blood products.[1]

Despite the dramatic decline in the number of cases, malaria remains an important health concern in the United States and other developed countries because international travelers import the disease. However, because the diagnosis of malaria requires considerable technical expertise and most laboratories infrequently receive requests to test for malaria, experts have raised concerns that laboratories may lack proficiency in detecting this parasite. Studies in Canada[2] and the United Kingdom[3] appear to validate this concern. However, we are unaware of any studies that have examined the performance of laboratories in the United States.

This study assesses the ability of laboratories in the United States to diagnose malaria. To do this, we examined both proficiency test (PT) performance and malaria screening practices of laboratories enrolled in American Proficiency Institute's (API) Parasitology program. The following is a report of our findings.

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Abbreviations

PT, proficiency test; API, American Proficiency Institute; RDT, rapid diagnostic test

Materials and Methods

We analyzed data from API's Parasitology proficiency testing program spanning the years 1999-2008. Participants in this program were laboratories in hospitals with 25-300 beds, and the number of participants in each PT event ranged from 15-84 over the 10-year period.

Each year, participants received 3 Giemsa-stained thin blood smears that were to be examined for blood parasites (ie, 1 blood smear in each of 3 PT events per year). Blood smears were obtained from 3 sources: Alexon-Trend (Ramsey, MN; merged with Remel) during the years 1999-2002, Remel (Lenexa, KS) during the years 2003-2007, and Meridian Bioscience (Cincinnati, OH) in 2008. Of the 30 smears evaluated during the 10-year period, 6 contained *P falciparum*, 7 contained *P malariae*, 1 contained *P ovale*, 3 contained *P vivax*, and 6 contained no parasites. Finally, 7 blood smears contained parasites other than *Plasmodium* sp.; these were excluded from our analysis.

All slides were evaluated by referee laboratories, and all results agreed with the expected results of the surveys. The manufacturers' Certificates of Analysis for 2 of the 3 *P vivax* specimens (years 2000 and 2004) and the *P ovale* specimen confirmed the presence of Shuffner's dots. However, the manufacturer's Certificate of Analysis for the *P vivax* specimen in 2008 did not mention the presence of Shuffner's dots, and none of the Certificates of Analysis for the *P falciparum* specimens explicitly noted the presence of gametocytes.

We analyzed the participant data in the following 3 ways:

1. We calculated the annual and 10-year cumulative percentages of unacceptable responses for specimens containing *Plasmodium* sp. Because many laboratories refer positive malaria specimens to a reference laboratory for definitive identification, acceptable results included the responses "*Plasmodium* sp," "Parasites seen, referred," and (if appropriate) "*Plasmodium*, not *falciparum*" in addition to correct identification of the organism.
2. We examined responses from laboratories that identified the species of *Plasmodium* and calculated the number and percentage of incorrect responses for each species.
3. We calculated the number and percentage of unacceptable responses for specimens containing no parasites.

To assess current laboratory practices, we faxed a questionnaire to 88 laboratories enrolled in API's Parasitology program in 2010. All of these laboratories were in hospitals with 25-300 beds. The questions were designed to elicit information about the extent of malaria testing performed in-house or offered via referral, frequency of requests for malaria screening, methods used, and turnaround time.

Results

Table 1 summarizes laboratories' performance in correctly detecting and identifying *Plasmodium* sp. Overall, 8.6% of responses were unacceptable. Performance was best in detection and identification of *P falciparum* (3.4% unacceptable responses), followed by *P vivax* (9.0% unacceptable responses), *P malariae* (10.9% unacceptable responses), and *P ovale* (20.6% unacceptable responses).

Rates of unacceptable responses were higher among laboratories that definitively identified the species of *Plasmodium* (**Table 2**). Overall, 21.2% of responses were unacceptable. Laboratories performed best at identifying *P falciparum* (11.2% unacceptable responses), followed by *P vivax* (21.7% unacceptable responses), *P malariae* (22.5% unacceptable responses), and *P ovale* (100.0% unacceptable responses).

During the study period, 6 PT specimens contained no blood parasites. The annual rates of unacceptable responses to these specimens ranged from 4.0%-11.3%, with an overall rate of 7.8% (**Table 3**).

Respondents to the laboratory practices survey included 36 laboratories performing some level of diagnostic testing for malaria in-house; **Table 4** summarizes their responses. Of these 36 laboratories, 22 perform all testing in-house, and their testing practices are as follows:

- Nine perform both species identification and parasitemia counts.
- Five perform species identification only; parasitemia counts are not offered.
- One performs parasitemia counts only; species identification is not offered.
- Seven perform neither species identification nor parasitemia counts.

The remaining 14 laboratories indicated they refer specimens for additional testing; their referral practices are as follows:

- Four refer specimens for both species identification and parasitemia counts.
- Five refer specimens for species identification; parasitemia counts are not offered.
- Three refer specimens for species identification; parasitemia counts are performed in-house.
- One refers specimens for parasitemia counts; species identification is performed in-house.
- One refers specimens for species identification but failed to answer the question about parasitemia counts.

Table 1_Failure to Detect or Misidentification of Malaria Parasites

	P falciparum		*P malariae*		*P ovale*		*P vivax*	
Year	Acceptable Results	Unacceptable Results	Acceptable Results	Unacceptable Results	Acceptable Results	Unacceptable Results	Acceptable Results	Unacceptable Results
1999	18	1	15	0	---	---	---	---
2000	45	4	---	---	---	---	42	0
2001	58	4	48	8	---	---	---	---
2002	---	---	52	6	---	---	---	---
2003	64	0	---	---	50	13	---	---
2004	---	---	82	2	---	---	79	3
2005	---	---	---	---	---	---	---	---
2006	76	3	69	10	---	---	---	---
2007	82	0	76	8	---	---	---	---
2008	---	---	60	15	---	---	61	15
Total	**343**	**12**	**402**	**49**	**50**	**13**	**182**	**18**
% Unacceptable (by species)		**3.4%**		**10.9%**		**20.6%**		**9.0%**
% Unacceptable (all combined)		**8.6%**						

Table 2_Misidentification of *Plasmodium* sp.

	P falciparum		*P malariae*		*P ovale*		*P vivax*	
Year	Acceptable Results	Unacceptable Results	Acceptable Results	Unacceptable Results	Acceptable Results	Unacceptable Results	Acceptable Results	Unacceptable Results
1999	2	1	1	0	---	---	---	---
2000	11	4	---	---	---		10	0
2001	16	4	9	0	---	---	---	---
2002	---	---	10	1	---	---	---	---
2003	26	0	---	---	0	12	---	---
2004	---	---	8	2	---	---	6	3
2005	---	---	---	---	---	---	---	---
2006	20	3	15	4	---	---	---	---
2007	20	0	6	3	---	---	---	---
2008	---	---	13	8	---	---	2	2
Total	**95**	**12**	**62**	**18**	**0**	**12**	**18**	**5**
% Unacceptable by species		**11.2%**		**22.5%**		**100.0%**		**21.7%**
% Unacceptable overall		**21.2%**						

Table 3_False Positive Proficiency Test Results for *Plasmodium* sp.*

Year	Acceptable Results	Unacceptable Results	% Unacceptable Results
1999	---	---	---
2000	---	---	---
2001	---	---	---
2002	56	6	9.7%
2003	---	---	---
2004	71	9	11.3%
2005	74	4	5.1%
2006	72	3	4.0%
2007	74	6	7.5%
2008	66	7	9.6%
Total	**413**	**35**	
% Unacceptable 1999-2008:		**7.8%**	

**No negative slides were sent to participants in 1999, 2000, 2001, and 2003.*

Table 4_Practices in Laboratories That Perform Malaria Testing

Survey Question	No. of Responses
How many blood films do you examine each month?	
Less than 1	30
1-5	4
6-10	1
More than 10	1
What method(s) do you use?	
Thin film only	6
Thick film only	3
Thick and thin film	26
Thick and thin film, RDT*	1
Do you identify the species?	
Yes	15
No	8
Send to reference laboratory	13
Do you perform parasitemia counts?**	
Yes	13
No	17
Send to reference laboratory	5
What is the TAT† for a preliminary result?§	
Less than 6 hours	22
6-12 hours	11
12-24 hours	2
More than 24 hours	0
What is the TAT for a final result?	
Less than 12 hours	4
12-24 hours	19
24-48 hours	6
More than 48 hours	7

**Rapid diagnostic test*
***One respondent did not answer this question.*
†Turnaround time
§One respondent provided 2 responses to this question; these were excluded.

In addition to the 36 laboratories screening for malaria in-house, 5 respondents indicated that they send specimens submitted for malaria screening to a reference laboratory for all testing. Because these respondents answered only the questions about turnaround time, their answers are not included in **Table 4**. In these 5 laboratories, turnaround time for a preliminary result was more than 24 hours for 4 laboratories and 12-24 hours for 1 laboratory. Turnaround time for a final result was more than 48 hours for 3 laboratories and 24-48 hours for 2 laboratories.

Discussion

Proficiency Testing Results

In this retrospective review, the rate of unacceptable responses to PT samples containing *P falciparum* was substantially lower than results reported in 2 previous studies.[2,3] Thomson and colleagues reported that 27% of laboratories in Ontario, Canada, failed to correctly diagnose *P falciparum*.[2]

A similar study by Milne and colleagues found that 21% of specimens with *P falciparum* were misdiagnosed by British laboratories.[3] By contrast, our data showed a failure rate of only 3.4% among all laboratories surveyed (ie, both laboratories that identify species and laboratories that screen for parasites and then refer positive specimens). In laboratories that identified the species, the failure rate rose to 11.2%, which is still substantially lower than the rates reported in the Canadian and British studies. Instead, our results more closely resemble data from the Malaria Parasite Quality Assurance Programme in Hong Kong, which reported failure rates of 5% or less for identification of *P falciparum* in a 5-year retrospective review.[4]

In contrast to their performance with *P falciparum*, participants had much more difficulty diagnosing *P malariae*, *P ovale*, and *P vivax*. Performance was especially poor in laboratories providing a definitive identification, with failure rates of 22.5% (*P malariae*), 21.7% (*P vivax*), and 100.0% (*P ovale*). The fact that none of the laboratories correctly identified *P ovale* is especially troubling. Whether this truly indicates laboratories have exceptional difficulty identifying this species is uncertain, because only 1 PT sample in the 10-year period contained this organism and only 12 respondents provided a species identification. However, both the British and Hong Kong studies reported that participants had more difficulty identifying *P ovale* than other *Plasmodium* sp.[3,4]

Although none of the laboratories correctly identified *P ovale*, 8 of the 12 respondents misidentified the organism as *P vivax*, and 4 misidentified it as *P malariae*. Mistakenly identifying *P ovale* as *P vivax* would be less likely to harm a patient, since treatment for both *P ovale* and *P vivax* includes primaquine. Nevertheless, the poor performance depicted in our data and the difficulties noted in the British and Hong Kong studies suggest a closer examination of the laboratories' ability to identify *P ovale* is warranted.

Failure rates for blood films containing no parasites ranged from 4.0%-11.3%, with an average of 7.8% over the 10-year period. These rates are substantially higher than the 2.0% false positive rate reported in the Canadian study.[2] Our results suggest laboratories may often incorrectly report the presence of parasites, which could lead to unnecessary treatment or a delayed diagnosis of the true cause of illness.

Malaria Screening Practices

We evaluated the responses to the Malaria Practices Survey using 4 criteria:

1. Initial screening should be performed immediately.
2. Final results should be available within 24 hours.
3. Both thick and thin blood films should be examined.
4. Testing should include both species identification and a parasitemia count.

Most laboratories met the goals of performing initial screening immediately and providing final results within 24 hours. Of the 35 respondents that answered the question about turnaround time for a preliminary result, 22 (62.8%) claimed a turnaround time of less than 6 hours. Another 11 (31.4%) reported a turnaround time of 6-12 hours. Fewer laboratories met the goal for a final result. Of 36 respondents, 23 (63.9%) claimed that final results are available within 24 hours. However, 13 (36.1%) failed to meet this goal, and 7 (19.4%) reported a turnaround time of more than 48 hours for a final report.

The use of both thick and thin Giemsa-stained blood films is the "gold standard" method to screen for malaria parasites.[5,6] Of the 36 laboratories responding to our survey, 9 (25.0%) failed to meet this standard. Of these, 3 screen only a thick film, and 6 screen only a thin film. All 3 of the laboratories screening only thick films and 4 of the laboratories screening only thin films also reported they screen less than 1 specimen per month. The remaining 2 laboratories reported volumes of 1-5 and 6-10 requests per month.

A complete diagnostic workup for malaria includes both an identification of the *Plasmodium* species and an estimate of the parasite burden (at least for *P falciparum*), because clinicians need this information to choose the most effective therapy for the patient. In our survey, only half of the respondents indicated their laboratories offer a complete diagnostic workup. The remaining 18 (50.0%) laboratories fail to offer species identification, parasitemia counts, or both:

- Eleven (30.6%) perform species identification (either in-house or by referral) but do not offer parasitemia counts.
- Six (16.7%) perform neither species identification nor parasitemia counts.
- One (2.8%) performs parasitemia counts but does not identify the species.

Recommendations

Based on our review of PT data and the responses to the Malaria Practices Survey, we recommend 4 steps laboratories can take to improve diagnosis of malaria:

1. Design educational initiatives to improve identification of *Plasmodium* sp and reduce false positive results. Our PT data suggest that laboratories are most proficient at identifying *P falciparum* and least proficient at identifying the remaining 3 species, so educational efforts should focus on *P malariae*, *P vivax*, and, especially, *P ovale*. Also, the high false negative rate in our study suggests that laboratory professionals would benefit from instruction to teach them to distinguish parasites from artifacts.
2. Ensure that requests for malaria screening are processed immediately. If specimens are referred for testing, choose a reference laboratory providing results quickly. Ideally, a final result should be available within 24 hours, but this is not always feasible. If the report cannot be finalized within 24 hours, the clinician should at least be informed of the presence of malaria parasites and provided a preliminary identification if possible.
3. Screen both thick and thin Giemsa-stained blood films for malaria parasites. Thick films are preferred for initial screening because they contain a greater amount of blood than thin films and are therefore more likely to contain parasites. Thin films are preferred for species identification because they allow better visualization of the parasite. If a laboratory lacks personnel with sufficient technical expertise, consider whether some or all of the testing should be submitted to a reference laboratory.
4. Consider adding a rapid diagnostic test (RDT) to the malaria screening protocol. In the responses to our Malaria Practices Survey, only 1 laboratory used RDT as part of its screening practices. This suggests that many laboratories may be unaware of the potential benefits of this relatively new testing method. Studies have shown RDTs may be useful in laboratories lacking technical expertise in microscopy,[5] and the CDC states RDT can improve the

rapid diagnosis of malaria in some health care settings.[7] Currently, only the BinaxNOW Malaria (Inverness Medical Professional Diagnostics, Scarborough, ME) is approved for use in the United States.[7] If RDT is used, it should always be followed by conventional examination of thick and thin films, because RDT does not give quantitative information about the parasite burden and it may not detect *P malariae*, *P ovale*, or mixed infections.

Conclusion

Even though malaria is now rare in the United States, it can occur in any region of the country as a result of international travel. Also, malaria can quickly become life threatening, especially if the patient is infected with *P falciparum*. For these reasons, it is essential that all hospital laboratories are able to quickly detect and identify malaria parasites, either by in-house testing or by referral. Testing protocols should ensure the specimen is examined immediately, the *Plasmodium* species is identified, a parasitemia count is performed at least for *P falciparum*, and final results are available within 24 hours. LM

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