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EVALUATION OF THE BINAX NOW® ICT TEST VERSUS POLYMERASE CHAIN REACTION AND MICROSCOPY FOR THE DETECTION OF MALARIA IN RETURNED TRAVELERS

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Abstract. Microscopic detection of *Plasmodium* species has been the reference standard for the diagnosis of malaria for more than a century. However, maintaining a sufficient level of expertise in microscopic diagnosis can be challenging, particularly in non-endemic countries. The objective of this study was to compare a new rapid malaria diagnostic device (NOW® ICT Malaria Test; Binax, Inc., Portland, ME) to polymerase chain reaction (PCR) and expert microscopy for the diagnosis of malaria in 256 febrile returned travelers. Compared with PCR, the NOW® ICT test showed a sensitivity of 94% for the detection of *P. falciparum* malaria (96% for pure *P. falciparum* infection) and 84% for non-*P. falciparum* infections (87% for pure *P. vivax* infections and 62% for pure *P. ovale* and *P. malariae* infections), with an overall specificity of 99%. The Binax NOW® ICT may represent a useful adjunct for the diagnosis of *P. falciparum* and *P. vivax* malaria in febrile returned travelers.

INTRODUCTION

In 2001 approximately 50 million people from western countries visited malaria-endemic areas and at least 30,000 travelers contracted malaria.[1] Despite treatment, between 1% and 4% of travelers who acquire *Plasmodium falciparum* malaria will die as a result of infection.[1] This fatality rate increases to 20% or higher in patients who develop severe malaria or those who are elderly.[2] Since 90% of travelers who contract malaria will not become ill until returning home, accurate diagnosis and appropriate treatment depend on the expertise of physicians and diagnostic laboratories in non-endemic areas.[3,4]

Although microscopic detection of parasites on Giemsa-stained blood smears has been the reference standard for malaria diagnosis in laboratories for more than a century, it is an imperfect standard highly dependent on the technical expertise of the microscopist.[5] The ability to maintain the required level of expertise in malaria diagnostics may be problematic especially in peripheral medial centers in countries where the disease is not endemic.[5] The World Health Organization has recognized the "urgent need for simple and cost-effective diagnostic tests for malaria to overcome the deficiencies of [both] light microscopy" and clinical diagnosis.[6] Consequently, recent efforts have focused on developing sensitive and specific non-microscopic malaria diagnostic devices including those based on the detection of malaria antigen in whole blood. Many first-generation rapid diagnostic products relied on the detection of the histidine-rich protein II (HRP II) antigen of *P. falciparum* and therefore could not detect other *Plasmodium* species. A newer generation of rapid diagnostic devices based on antigen capture with immunochromatographic (ICT) strip technology and use of monoclonal antibodies to HRP II for the detection of *P. falciparum* as well as aldolase, a pan-*Plasmodium* antigen, thus facilitating identification of non-falciparum infections. The performance of one device, the ICT Malaria P.f/P.v (AMRAD-ICT Diagnostics, Sydney, Australia), has been previously evaluated.[7–13] However, this device is no longer available.

A new rapid assay, the Binax, Inc. (Portland, ME) NOW® ICT malaria test, is designed to detect both falciparum and non-falciparum infections and may possess technical advantages over its predecessors. The objective of this study was to examine the performance of the NOW® ICT test compared with a blinded polymerase chain reaction (PCR) and expert microscopic analysis for the diagnosis of all human malaria species in febrile travelers returning from malaria-endemic areas.

MATERIALS AND METHODS

Patients. Patients presenting to the Tropical Disease Unit of the Toronto General Hospital from July 1999 to January 2003 with fever ($\geq$ 38°C) or a history of fever (within 48 hours) and travel to a malaria-endemic area were eligible for inclusion in the study. All patients with blood films containing malaria parasites were enrolled. In addition, patients who had repeatedly negative blood films during the first two months of the study, (i.e., diagnosed with a febrile illness other than malaria) were enrolled to provide a comparable control group. The prevalence of malaria in returned travelers during the study period was 15%.

Whole blood samples (pretreatment) were collected from all patients for thick and thin blood film preparation, PCR, rapid diagnostic tests, and complete blood counts. An expert microscopist who was blinded to the results of additional diagnostic testing examined the blood films. Smears were considered negative if no parasites were seen in 500 oil-immersion fields (1,000×) on a thick blood film. Parasite concentration was calculated by determining the number of parasites per 200 or 500 white blood cells in a thick blood film. Baseline white blood cell counts were used to calculate parasitemia (parasites per microliter). All PCR amplification, species identification, and diagnostic assays were performed in a blinded fashion. This study was reviewed and approved by the Ethical Review Committee of the University Health Network-Toronto General Hospital.

Polymerase chain reaction. Detection and malaria species identification by PCR were performed as previously described.[14–16] Briefly, genomic DNA was extracted from whole blood samples using Qiagen columns (Qiagen, Chatsworth, CA) following the manufacturer's instructions. A

5-μL aliquot of the DNA extract was used in a nested PCR assay to amplify a segment of the *Plasmodium* 18S ribosomal RNA gene. The resulting PCR product was analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide as previously described.[15]

NOW® ICT assay. The NOW® ICT Malaria Test for Whole Blood is a rapid, *in vitro* immunodiagnostic test for the detection of circulating *P. falciparum* antigen and a pan-malaria antigen in whole blood. The test card contains immobilized antibodies specific for the HRPII antigen of *P. falciparum* and antibodies specific for aldolase, a pan-malaria antigen. The assays were performed according to the manufacturer's instructions. The test results were independently examined and interpreted by three observers blinded to the microscopic and PCR results. The final results of the test were recorded as either negative or positive based on the majority agreement. The readers also graded the assays results (as band intensity for the HRP II and *pan-Plasmodium* antigen bands) ranging from 0 (negative: no visible reaction for either HRP II or pan-malaria antigen) to 4+ (strongly positive reaction for both or either antigen) (Figure 1).

Data analysis. The sensitivity and specificity of the Binax NOW®ICT test were calculated with PCR results as the reference standard. Positive and negative predictive values were calculated based on the prevalence of malaria infections in all patients presenting to the Tropical Disease Unit of the Toronto General Hospital during the study period. The *K* statistic was used to measure agreement among the three independent observers.

RESULTS

During the study period, 256 individuals who presented with fever after travel to a malaria- endemic area were enrolled. The ratio of male to female patients was 1.6 (63.5% males and 36.5% females) with a mean ± SD age of 32 ± 1.1 years (range = 4 months to 71 years). Travel destinations included Africa (58.1%), the Indian Subcontinent (17%), Latin America (17%), Oceania (3.8%), Southeast Asia (2.9%), and the Middle East (0.9%). Whole blood samples from 101 of these individuals were confirmed by PCR to be positive for *P. falciparum* malaria, 90 were PCR confirmed *P. vivax* infections, 9 were *P. ovale,* 3 were *P. malariae,* 6 were mixed infections, and 47 were PCR and blood smear negative. Malaria-infected patients did not differ significantly from other patients with respect to age, sex, or duration of illness.

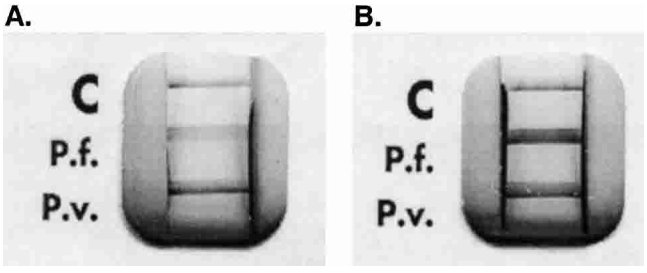

FIGURE 1. Binax ICT result showing **A**, low *Plasmodium falciparum* parasitemia (band intensity of 1+) and **B**, high *P. falciparum* parasitemia (band intensity of 4+). C = control band; P.f. = *Plasmodium falciparum*; P.v. = *Plasmodium vivax.*

The results of the NOW® ICT test compared with PCR-based diagnosis are shown in Table 1. The results of the NOW® ICT test compared with microscopic diagnosis are shown in Table 2.

When compared with the PCR, the sensitivity of the NOW® ICT assay was 95.5% for the detection of pure *P. falciparum* infections, 94.3% for *P. falciparum* when present either pure or as a mixed infection (i.e., *P. falciparum* mixed with *P. vivax*, *P. ovale*, or *P. malariae*), 86.7% for pure *P. vivax* infections, and 83.5% for all non-falciparum infections. For pure *P. ovale* and *P. malariae* infections, the test had a sensitivity of 61.5%. The overall specificity of the text was 98.7%. Based on a malaria prevalence of 15% during the course of the study, the corresponding positive predictive value for *P. falciparum* and non-*P. falciparum* infections were 89.8% and 88.4% and the negative predictive values were 97.7% and 93.8%, respectively. There was excellent agreement between the three independent observers, with a K value of 0.99 for the *P. falciparum* line and 0.94 for the pan-malaria line. When microscopy was used as a reference standard (Table 2) the corresponding sensitivity was 96.0% and 84.7% for falciparum and non-falciparum infections respectively, with a specificity of 98.7%.

When compared with the PCR, the NOW® ICT test yielded two false-positive and 23 false-negative results (Table 1), with nearly perfect agreement among all three independent observers. Compared with microscopy, the NOW® ICT test yielded 19 false-negative results, of which 15 occurred in specimens with parasitemias < 1,000 parasites/μL. The sensitivity of the rapid assay compared with parasitemia for *P. falciparum* and *P. vivax* infections are shown in Tables 3 and 4. The sensitivity of the assay decreased to 75% for falciparum infections with parasitemias < 100 parasites /μL (Table 3) and to ≤ 55% for vivax infections with parasitemias < 1000 parasites/μL (Table 4).

DISCUSSION

In this study, we examined the performance characteristics of the Binax NOW® ICT rapid malaria diagnostic device using PCR as the primary reference standard based on its established advantages over microscopy, particularly in cases of low parasitemia and in mixed infections.[3,5,14,15,17–20] Our results indicate that the NOW® ICT test is sensitive (94.3%) and specific (98.7%) for the detection of *P. falciparum* malaria in returned travelers. The assay was also specific but somewhat less sensitive (83.5%) for the detection of non-falciparum malaria (86.7% for pure *P. vivax* infections). The

TABLE 1

Results of the NOW® ICT test compared with polymerase chain reaction (PCR) as the reference standard for the diagnosis of malaria in febrile returned travelers

NOW® ICT result	No. of samples with indicated PCR results			
	*P. falciparum**	Non-*P. falciparum*†	Negative	Total
Positive	100	86	2	188
Negative	6	17	45	68
Total	106	103	47	256

* Includes *Plasmodium falciparum* mixed infections with *P. vivax, P. ovale,* and *P. malariae.*

† *P. vivax, P. ovale,* and *P. malariae* single and mixed infections.

TABLE 2

Results of the NOW® ICT test compared with expert microscopy as the reference standard for the diagnosis of malaria in febrile returned travelers

NOW® ICT result	No. of samples with indicated smear results*			
	P. falciparum†	Non-*P. falciparum*‡	Negative	Total*
Positive	97	83	2	182
Negative	4	15	45	64
Total	101	98	47	246

* There were 10 samples in which reliable identification to the species level by microscopy was not possible, but which were identified as *Plasmodium falciparum* (6) and *P. vivax* (4) by a polymerase chain reaction. These samples were excluded from analyses using microscopy as the reference standard.
† Includes *P. falciparum* mixed infections with *P. vivax, P. ovale,* and *P. malariae.*
‡ *P. vivax, P. ovale,* and *P. malariae* single and mixed infections.

sensitivity for the detection of pure *P. ovale* and *P. malariae* infections was 61.5% with a specificity of 100%. The expression of the *pan-Plasmodium* antigen in *P. ovale*, and more so in *P. malariae*, has not been fully characterized,[13,21] and this combined with low parasitemias in these infections likely accounts for relatively lower sensitivity for the detection of these malaria species. It is important to note that this test does not distinguish between the non-falciparum species (*P. vivax*, *P. ovale*, and *P. malariae*), nor can it reliably distinguish pure *P. falciparum* infections from mixed falciparum infections.

The test is simple to perform, rapid (< 15 minutes), and easy to interpret, with excellent inter-reader agreement (K value = 0.99). A K value > 0.81 indicates almost perfect agreement between observers. Discrepancies between readers occurred mainly when the test result was weakly positive, most frequently when the sample had a low parasitemia. A weak correlation (r = 0.359) was observed between the intensity of the *P. falciparum* band and parasitemia, but a slightly stronger correlation (r = 0.637) was observed with the intensity of the *pan-Plasmodium* band.

Rapid diagnostic assays may be most useful when expert microscopy is not available. Although microscopy can be sensitive to a threshold of 5–50 parasites/μL, depending on the expertise of the microscopist and equipment limitations,[12] the average microscopist is likely to achieve a sensitivity closer to 100 parasites/μL or higher. In this investigation, the sensitivity of the NOW® ICT test for *P. falciparum* infections was > 95% for samples with > 100 parasites/μL and > 94% for *P. vivax* infections for parasitemias > 1,000 parasites/μL; however, for both falciparum and vivax infections sensitivity decreased as parasitemia decreased.

False-negative results, particularly for *P. falciparum*, are of concern. Five false-negative results did occur for *P. falciparum* infections (four with < 600 parasites/μL and one with > 10,000 parasites/μL), 12 for *P. vivax* (nine with < 700 parasites/μL and three with < 7,300 parasites/μL), five for *P. ovale* (all with < 600 parasites/μL), and one for a *P. falciparum*, *P. ovale*, *P. malariae* mixed infection in which only the *P. falciparum* infection was detected (520 parasites/μL). The observation that false-negative results may occasionally occur even at high parasitemia is of concern. Previous studies of HRP II-based assays have reported the same limitation and potential explanations include a prozone effect (i.e., a high concentration of antibody may mask the antigen making it unavailable to be detected in these rapid assays) or the presence of a mutation or deletion within the *hrp ii* gene.[14] Thus, when the clinical suspicion of malaria remains high despite a negative rapid diagnostic test result, the assay should be repeated within 12–24 hours[14] and these assays should be performed in parallel with thick and thin blood smears.

TABLE 3

Binax Now® ICT assay for the detection of *Plasmodium falciparum* malaria according to the level of parasitemia

Parasitemia (no. of parasites/μL of whole blood)	Microscopy (no. positive)	NOW ICT (no. positive)	Sensitivity (%)
1–100	4	3	75.0
101–1,000	26	25	96.2
1,001–10,000	37	36	97.3
>10,000	34	33	97.1

TABLE 4

Binax Now® ICT assay for the detection of *Plasmodium vivax* malaria according to the level of parasitemia

Parasitemia (no. of parasites/μL of whole blood)	Microscopy (no. positive)	NOW ICT (no. positive)	Sensitivity (%)
1–100	6	3	50.0
101–1,000	20	11	55.0
1,001–10,000	51	48	94.1
>10,000	21	21	100

There were two false-positive NOW® ICT tests results in this study. Occasional false-positive results due to the presence of rheumatoid factor have previously been reported with diagnostic devices based on the detection of HRP II.[22] Furthermore, detection of antigen may persist for up to 28 days after cure of infection.[18]

Despite some inherent limitations, evidence suggests that rapid malaria diagnostic devices might represent a useful diagnostic adjunct tool to microscopy in a clinical setting. Since laboratories in areas where malaria is not endemic frequently lack expertise in diagnostic microscopy, a rapid diagnostic assay could provide a quick and accurate although still preliminary diagnosis, while definitive results are sought from a reference laboratory. Importantly, due to the occurrence of occasional false-negative results with rapid diagnostic assays, malaria infection cannot be ruled out based on a negative result. Microscopy remains essential for species identification, parasitemia calculations, as well as a backup to exclude false-negative results.

In conclusion, the Binax NOW® ICT malaria test is a rapid and easy to use diagnostic assay. The test achieves high specificity (> 95%) for all *Plasmodium* species and high sensitivity for *P. falciparum* infections, but is less sensitive for the detection of non-falciparum malaria species, especially at parasitemias < 1000 parasites/μL. Further studies are necessary to establish the field performance of the NOW® ICT assay.

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