

Evaluation of the performance of the ABON™ Plus Malaria® and I-test® tests in the diagnosis of imported malaria in Tunisia

Evaluation des performances des tests ABON™ Plus Malaria® et I-test® dans le diagnostic du paludisme d'importation en Tunisie

Dorsaf Aloui, Oumaima Khemis, Mariem Bouchakoua, Sonia Trabelsi

University of Tunis El Manar, Faculty of Medicine of Tunis, 1007, Charles Nicolle Hospital, Laboratory of Parasitology-Mycolology, Tunis, Tunisia

ABSTRACT

Background: Imported malaria remains a public health challenge in malaria-free countries. Rapid diagnostic tests (RDTs) are used alongside microscopy, but their performance varies with clinical presentation and epidemiological context.

Methods: We conducted a retrospective study (2020–2023) at the Parasitology-Mycolology Laboratory of Charles Nicolle Hospital, Tunisia, including 25 symptomatic patients and 246 asymptomatic non-permanent resident students from sub-Saharan Africa. Microscopy (thick and thin smears) served as the reference standard. Sensitivity, specificity, positive and negative predictive values (PPV, NPV), and Cohen's kappa (κ) were calculated to assess the performance of the ABON™ PLUS Malaria (Abon biopharm, China) et I-test® (Bacterovir laboratoire) RDTs.

Results: Malaria was diagnosed in 35 individuals (13%), comprising 12 symptomatic patients (48%) and 23 asymptomatic patients (9.35%). *Plasmodium falciparum* was the most common species (95%).

In symptomatic subjects, parasitemia ranged from 0.5% to 20%, and both tests demonstrated excellent agreement with the thick blood smear ($\kappa=0.91$ for ABON™ Plus; $\kappa=1$ for I-test®). In contrast, parasitemia was low ($<0.1\%$) in asymptomatic students. For this group, the ABON™ Plus test showed a sensitivity of 48% and a specificity of 100% (PPV: 100%; NPV: 95%), while the I-test® had a sensitivity of 23.8% and a specificity of 100% (PPV: 100%; NPV: 90%). Consequently, agreement with the reference smear was good for the ABON™ Plus test ($\kappa=0.62$) but poor for the I-test® ($\kappa=0.35$).

Conclusion: Both RDTs performed well in symptomatic cases, but low sensitivity in asymptomatic individuals highlights the importance of microscopy and targeted screening to prevent malaria reintroduction in malaria-free countries.

Keywords: Imported malaria, Rapid diagnostic tests, screening, Tunisia

RÉSUMÉ

Contexte : Les tests de diagnostic rapide (TDR) du paludisme sont utilisés avec la microscopie, mais leurs performances varient selon le contexte clinique et la parasitémie.

Méthodes : Nous avons mené une étude rétrospective entre 2020 et 2023 au laboratoire de parasitologie de l'hôpital Charles Nicolle à Tunis. L'étude a porté sur 25 patients symptomatiques et 246 étudiants asymptomatiques non-résidents permanents. La microscopie était la méthode de référence. Nous avons évalué les performances des TDR ABON™ Plus Malaria (Abon Biopharm, Chine) et I-test® (Bacterovir laboratoire).

Résultats : En total de 35 cas de paludisme (13 %) a été diagnostiqué : 12 chez les patients symptomatiques (48 %) et 23 chez les étudiants asymptomatiques (9,35 %). *Plasmodium falciparum* représentait 95 % des infections. Chez les sujets symptomatiques, la parasitémie variait de 0,5 % à 20 %, et les deux tests ont montré une excellente concordance avec la microscopie (coefficient $\kappa = 0,91$ pour ABON™ Plus et coefficient $\kappa = 1$ pour I-test®). Chez les individus asymptomatiques, la parasitémie était faible ($<0,1\%$). Dans ce groupe, ABON™ Plus affichait une sensibilité de 48 % et une spécificité de 100 %, tandis que I-test® présentait une sensibilité de 23,8 % pour une spécificité de 100 %. La concordance était modérée pour ABON™ Plus (coefficient $\kappa = 0,62$) et faible pour I-test® (coefficient $\kappa = 0,35$).

Conclusion : Ces résultats montrent que les TDR sont performants chez les sujets symptomatiques, mais leur faible sensibilité en cas d'infections pauciparasitémiques confirme le rôle indispensable de la microscopie dans le dépistage du paludisme d'importation.

Mots clés : Paludisme d'importation, test de diagnostic rapide, dépistage, Tunisie

Correspondance

Oumaima Khemis

University of Tunis El Manar, Faculty of Medicine of Tunis, 1007, Charles Nicolle Hospital, Laboratory of Parasitology-Mycolology, Tunis, Tunisia

Email: oumaymakhemis@gmail.com

What is known

- Imported malaria remains a diagnostic challenge in non-endemic countries, particularly in returning travelers and asymptomatic individuals.
- Rapid diagnostic tests (RDTs) are widely used for malaria diagnosis, but their performance may vary according to the *Plasmodium* species and parasite density.

What this article adds

- This study evaluates the diagnostic performance of ABON™ Plus Malaria® and I-test® in imported malaria cases diagnosed in Tunisia.
- It compares the sensitivity and specificity of these RDTs in both symptomatic patients and asymptomatic international students from endemic areas.
- The findings highlight the strengths and limitations of these tests and support their role as complementary tools to microscopy in non-endemic settings.

INTRODUCTION

Malaria continues to be one of the most devastating parasitic infections worldwide (1).

According to the World Health organization (WHO), an estimated 263 million malaria cases and 597,000 related deaths occurred in 2023. Africa registers 94% of malaria cases worldwide and 95% of malaria-related deaths. Within the region, children under five remain the most vulnerable, making up nearly three-quarters (76%) of fatalities (1).

Although Tunisia is officially malaria-free, imported cases constitute a potential reservoir for the parasite and represent a real risk of reintroduction, particularly among travelers and students from endemic areas (2,3). Since 1996, the national prevention strategy has been based on enhanced epidemiological surveillance. The strategy integrates systematic screening of at-risk populations with rapid diagnosis and effective treatment to limit local transmission (3,4).

Traditionally, malaria diagnosis has relied on microscopy, with thick and thin blood smears regarded as the gold standard because of their sensitivity, species identification, and ability to quantify parasitemia (1). Microscopy requires trained staff and labs, while rapid diagnostic tests (RDTs) rapidly detect *Plasmodium* antigens as a practical complement.

The aim of this study was to evaluate the performance of two RDTs, ABON™ PLUS Malaria (Abon biopharm, China) et I-test® (Bacterovir laboratoire), compared to microscopy. It focused on the diagnosis of imported malaria in symptomatic travelers and the systematic screening of students who are not permanent residents of Tunisia, in order to provide relevant data for the surveillance and prevention of this disease in a country that is officially malaria-free.

METHODS

We conducted a retrospective descriptive study at the Parasitology-Mycology Laboratory of Charles Nicolle Hospital in Tunis. It covered all cases of malaria recorded during three academic years, from 2020 to 2023.

The study population included Tunisian and foreign patients referred to the laboratory with suspected malaria, whose parasitological diagnosis was confirmed, and the non-permanent resident students of Tunisia (NPRST) from endemic areas, who were systematically screened as part of the national surveillance program. Individuals with negative *Plasmodium* test results were excluded from the study. Ethics Committee approval was not required for this research because it involved only a retrospective review of anonymized laboratory records, without any direct interaction with human subjects or the collection of identifiable data. The study complies with the principles of the World Medical Association Declaration of Helsinki.

Parasitological diagnosis was based on Giemsa-stained thick and thin blood smears. Thick blood smears were used to confirm infection, while thin smears identified *Plasmodium* species, developmental stage, and parasitemia. Blood samples were collected in EDTA tubes and processed promptly to prevent parasite degradation. Parasitemia was expressed as the percentage of infected red blood cells among the total observed.

Two RDTs were evaluated alongside microscopy. The first, ABON™ Plus Malaria® (Abon biopharm, China), detects *P. falciparum* HRP2 and pan-malarial aldolase present in all four human *Plasmodium* species. The test works by capillary migration of whole blood, allowing antigens to bind monoclonal antibodies on a membrane. A control band validates the test, while the P.f and Pan bands indicate the infecting species, confirming *P. falciparum* and suggesting other or mixed infections. The second assay, I-test® (Bacterovir laboratoire), introduced in the laboratory in 2021, uses a similar immunochromatographic principle with colloidal gold conjugates. Interpretation follows the same criteria: P.f band alone or with Pan indicates *P. falciparum*, while an isolated Pan band indicates non-falciparum species. All data collected were analyzed using SPSS version 21.0 software.

The performance of the RDTs was assessed by calculating sensitivity, specificity, and positive and negative predictive values (PPV, NPV), with thick blood smears serving as the reference standard. The concordance between RDT and microscopy results was evaluated using Cohen's Kappa coefficient (κ).

RESULTS

During the study period, 271 cases were examined at the Parasitology-Mycology Laboratory, including 25 symptomatic patients and 246 asymptomatic NPRST. Malaria was confirmed in 35 cases (12.9%), all by thick smear and/or blood smear.

Most cases were male (62.9%, sex ratio = 1.69), with a mean age of 26.7 ± 8 years (range: 19–49), and the 20–24 age group being the most affected. Annual case distribution peaked during the 2021–2022 academic year (21 cases, 60%), followed by 6 cases (17%) in 2020–2021 and 8 cases (23%) in 2022–2023.

Infections were predominantly imported from sub-Saharan Africa (94.3%), with Côte d'Ivoire (20%) and Chad (14.3%) being the most frequent sources, followed by Gabon (11%) and Burkina Faso, Cameroon, and Guinea (8% each).

Among symptomatic patients, malaria was confirmed in 12 individuals, representing a prevalence of 48%, mostly men (sex ratio = 2), 5 of whom were Tunisian, with a mean age of 35 years. All had been exposed in sub-Saharan Africa, mainly in Côte d'Ivoire (42%) and Chad (25%). None of the Tunisian patients had undergone chemoprophylaxis, and three had a history of malaria. (Table 1)

Table 1. Summary of malaria cases

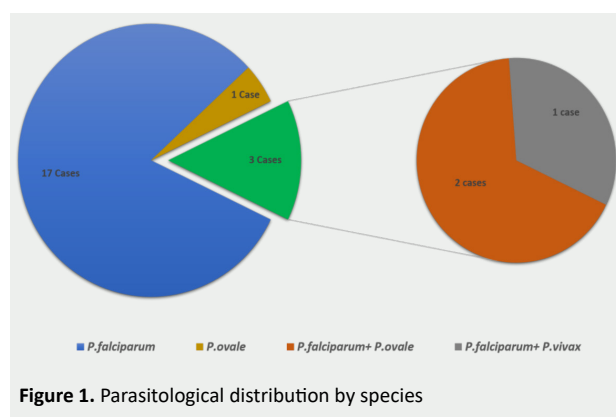
Group	Total number	Positive cases	Prevalence (%)	Mean age $\pm$ SD	Sex ratio (M/F)	Main source	History of malaria (%)
Symptomatic patients	25	12	48	35	2	Sub-Saharan Africa (Côte d'Ivoire 42 %, Chad 25 %)	25
Asymptomatic NPRST	246	23	9,35	$22,3 \pm 4$	1,3	Sub-Saharan Africa (Gabon, Cameroun)	43
Total	271	35	12,9	$26,7 \pm 8$	1,69	Sub-Saharan Africa (94,3 %)	–

NPRST: the non-permanent resident students of Tunisia

Among asymptomatic NPRSTs, 23 were parasitically infected (prevalence 9.35%), with the majority of cases occurring during the 2021–2022 academic year (63%). The mean age of infected NPRSTs was 22.3 ± 4 years, with a sex ratio of 1.3. Almost all infections originated in sub-Saharan Africa (91.3%), mainly Gabon and Cameroon. Among these students, 43% had a history of malaria. (Table 1)

Thick smear (TS) confirmed the diagnosis in 34 cases, while blood smear (BS) was positive in only 21 patients (11/12 symptomatic and 10/23 asymptomatic), corresponding to a sensitivity of 61.8% compared to TS. One particular case involved a febrile patient returning from an endemic area with two positive rapid tests but a negative microscopy result, probably due to self-medication with Artemether-Lumefantrine.

Identification by BS was possible in 21 cases, with a predominance of *P. falciparum* (95%, n=20) and one case of *P. ovale*. Three mixed infections were observed (2 *P. falciparum* + *P. vivax*, 1 *P. falciparum* + *P. ovale*). Parasitemia was very low (<0.1%) in asymptomatic students and ranged from 0.5% to 20% in symptomatic patients (mean 4.7%, median 3%) (Figure 1). (Table 2)



Of the 271 cases referred to our laboratory, all underwent at least one rapid diagnostic test (RDT) in addition to microscopic examination (TS and BS).

The ABON™ Plus Malaria® test was used in almost all patients, while the I-test® test, introduced in 2021, was performed in some of the cases.

In symptomatic patients, RDTs performed excellently: 10 of the 11 patients confirmed by TS were detected, corresponding to a sensitivity of 91% and high concordance (kappa = 0.83).

The ABON™ Plus Malaria® test had a sensitivity of 100%, a specificity of 92%, and excellent concordance (kappa = 0.91), while I-test® showed perfect concordance with microscopy (kappa = 1). (Table 3)

In asymptomatic NPRST, the overall sensitivity of RDTs was lower. Among the 23 cases confirmed by microscopy, 13 had a positive RDT, with a sensitivity of 56.5% and a specificity of 100% (kappa = 0.7). The ABON™ Malaria test, performed on all NPRSTs, detected 11 cases (sensitivity 48%, specificity 100%, kappa = 0.62), while the I-test®, used in 166 NPRSTs, detected only 5 cases among the 21 microscopically positive cases, with a sensitivity of 23.8%, specificity of 100%, and a poor kappa coefficient of 0.35 (Table 4).

Table 2. Parasitological profile and detection by rapid tests

Groupe	Patient	TS	BS	Plasmodial species	Parasitic stage	Parasitemia	ABON® Plus	I-test®	Other RDT
Symptomatic	1	+	+	P. ovale	Trophozoites	1%	NF	ND	OnSite -
	2	+	+	P. falciparum + P. ovale	Trophozoites (P.f+P.o)+ Gametocytes (P.f+P.o)	20%	+ (3 bands)	ND	ND
	3	+	+	P. falciparum + P. vivax	Trophozoites (P. f+P.v)	3%	+ (3 bands)	ND	OnSite+
	4	+	+	P. falciparum + P. vivax	Trophozoites(P.f+P.v) + Gametocytes(P. f+P.v) +Schizonts P.v	2%	+ (3 bands)	ND	OnSite+
	5	+	+	P. falciparum	Trophozoites + Gametocytes	4%	+ (2 bands)	ND	OnSite+
	6	+	+	P. falciparum	Trophozoites	3%	+ (2 bands)	ND	OnSite+
	7	-	-	-	-	-	+ (2 bands)	ND	OnSite+
	8	+	+	P. falciparum	Trophozoites	3%	+ (2 bands)	ND	ND
	9	+	+	P. falciparum	Trophozoites	1%	+ (2 bands)	+(2bands)	OnSite+
	10	+	+	P. falciparum	Trophozoites	0,5%	+ (2 bands)	ND	ND
	11	+	+	P. falciparum	Trophozoites	2%	+ (3 bands)	+ (3bands)	ND
	12	+	+	P. falciparum	Trophozoites	12%	+ (2 bands)	+ (2bands)	ND
NPRST	1	+	-	P. falciparum	Trophozoites + Gametocytes	<1%	+ (3 bands)	ND	-
	2	+	-	P. falciparum	Trophozoites	<0,1%	+ (3 bands)	ND	-
	3	+	-	-	-	-	-	-	-
	4	+	-	-	-	-	-	-	-
	5	+	-	-	-	-	-	-	-
	6	+	-	P. falciparum	Trophozoites	<0,1%	+ (3 bands)	-	-
	7	+	+	P. falciparum	Band P. f	-	+	-	-
	8	+	-	-	-	-	-	-	-
	9	+	-	-	-	-	-	-	-
	10	+	-	P. falciparum	Weak band	-	+	-	-
	11	+	-	P. falciparum	Trophozoites	<0,1%	+ (3 bands)	-	-
	12	+	-	P. falciparum	Trophozoites	<0,1%	+	-	-
	13	+	-	-	-	-	-	-	-
	14	+	-	P. falciparum	Trophozoites	<0,1%	+	-	-
	15	+	-	P. falciparum	Trophozoites	<0,1%	+	+	+
	16	+	-	-	-	-	-	+ (3bands)	-
	17	+	-	-	-	-	-	+ (Band P. f)	-
	18	+	-	-	-	-	-	-	-
	19	+	-	P. falciparum	Trophozoites	<0,1%	+	+	+
	21	+	-	-	-	-	-	-	-
	22	+	-	P. falciparum	Trophozoites	<0,1%	-	-	-
	23	+	-	P. falciparum	Trophozoites	<0,1%	+	+	+

BS: blood smears TS: Thick smear ND: not done RDT: Rapid diagnostic tests
P.f : Plasmodium falciparum P.o : Plasmodium ovale P.v : Plasmodium vivax

Table 3. Diagnostic performance of rapid tests (RDTs) in symptomatic patients

Test	TS (+) / Test (+)	TS (-) / Test (+)	TS (+) / Test (-)	TS (-) / Test (-)	Total	Sensitivity (%)	Specificity (%)	Kappa
RDT (global)	10	1	1	13	25	91,0	93,0	0,83
ABON™ Plus	10	1	0	12	23	100	92,0	0,91
I-test®	3	0	0	7	10	100	100	1

RDT: Rapid diagnostic tests TS: Thick smear

Table 4. Diagnostic performance of rapid tests (RDTs) in asymptomatic patients

Test	TS (+) / Test (+)	TS (-) / Test (+)	TS (+) / Test (-)	TS (-) / Test (-)	Total	Sensitivity (%)	Specificity (%)	Kappa
RDT (global)	13	0	10	223	246	56,5	100	0,70
ABON™ Plus	11	0	12	223	246	48,0	100	0,62
I-test®	5	0	16	145	166	23,8	100	0,35

RDT: Rapid diagnostic tests TS: Thick smear

Discussion

Our study identified 35 cases of malaria among 271 samples analyzed, representing an overall prevalence of 13%. This proportion varied according to the population studied: 48% among symptomatic patients (12 cases/25) and 9.35% among foreign students who were not permanent residents (23 cases/246). These results are consistent with Tunisian studies conducted at the Parasitology-Myecology Department of La Rabta Hospital. A 21-year study (1991–2012) of 430 screened NPRSTs revealed a positivity rate of 3% (5).

An earlier study conducted by Aoun et al. (1999–2006) on 2,478 asymptomatic students (NPRST, immigrant workers from malaria-endemic areas, and Tunisians returning from travel to endemic areas) as part of the national malaria control program reported only 42 cases, representing a prevalence of 1.7% (6).

A comparison of these different series highlights a growing trend in the frequency of imported malaria, reaching in our cohort the highest rate reported to date in Tunisia.

Malaria cases peaked in 2021–2022, over three times higher than in 2020–2021, before slightly declining in 2022–2023 while remaining above pre-pandemic levels. This pattern likely reflects the impact of COVID-19, including mobility restrictions and border closures in 2020–2021, followed by the resumption of international travel and disruptions to malaria prevention and treatment services in Africa, which contributed to increased in cases from 232 million in 2019 to 245 million in 2020, and a corresponding rise in mortality from 568,000 to 625,000 deaths (7).

Demographic analysis showed a male predominance (63%), which was more pronounced among symptomatic patients (sex ratio = 2), as reported in Tunisian and international studies on imported malaria (8). This male overrepresentation could be explained by increased exposure related to travel and activities in the countries of origin (9). The average age of patients was 26.7 years (range 19–49 years). Symptomatic patients were mainly aged 35–39 years, whereas asymptomatic students were predominantly 20–24 years old, consistent with previous Tunisian reports (10).

Most cases (85.7%) occurred in foreign nationals, while Tunisians represented 14.3% of infections, primarily among symptomatic patients (41.6%). Sub-Saharan Africa was the main region of contamination (94.3%), with a predominance of Côte d'Ivoire (20%) and Chad (14.3%). These results are consistent with the Tunisian context, where previous studies also show a predominantly West African origin: at the Parasitology-Myecology Department of La Rabta Hospital, 76.6% of cases came from this region, with Côte d'Ivoire and Mali in the lead (9). Worldwide, the WHO reports that six sub-Saharan African countries accounted for 55% of cases in 2020 (11). This comparison highlights the consistency of our study with national and international trends in imported malaria, confirming the central role of West Africa as an area of exposure.

None of the five Tunisian patients infected with parasites had undergone chemoprophylaxis, a phenomenon

already reported in several national studies (9). This non-compliance is thought to be linked to a lack of information about the severity of malaria and poor adherence due to the side effects of treatment.

According to the WHO, parasitological confirmation should ideally be obtained within two hours so as not to delay treatment. Microscopy of blood smears (BS) and thick smears (TS) remains the gold standard in terms of sensitivity and specificity.

In our study, among the 34 cases confirmed by TS, only 21 had a positive BS, giving an overall sensitivity of 61.8%. This sensitivity varied according to the population: 43.5% in asymptomatic students, probably due to very low parasitemia (<0.1%) in immune individuals, below the BS detection threshold (100–200 parasites/ μ L) (12). Conversely, BS was positive in all symptomatic patients, with parasitemia ranging from 0.5% to 20% (median 3%). Two non-immune Tunisian patients had parasitemia >4%, corresponding to a severity indicator according to the WHO.

The sensitivity of BS can also be influenced by the type of sample: capillary blood generally shows higher parasite densities than venous blood, which could reflect sequestration or deep migration of schizonts, particularly for *P. falciparum*, the predominant species in our asymptomatic BS-positive cases (12).

Among the 21 cases detected by BS, *P. falciparum* was the predominant species, accounting for 95.2% of infections. A single *P. ovale* case and three mixed infections including two involving *P. falciparum* and *P. vivax* were also reported. These findings are in line with Tunisian national data, which indicate that *P. falciparum* represents 86.8% of cases, while *P. vivax* and *P. ovale* together account for 10.2% (10). This predominance is also reported internationally, for example in the United States, where *P. falciparum* causes 69.8% of imported cases (13).

RDTs, developed in the 1990s to overcome the limitations of microscopy, can detect specific antigens from capillary or venous blood in 10 to 20 minutes, but do not measure parasitemia and are not a substitute for microscopy. They detect HRP2 (specific to *P. falciparum*), pLDH (pan-specific or specific to *P. falciparum* or *P. vivax*) and aldolase, whose rapid disappearance after treatment allows therapeutic efficacy to be monitored (14). Combined tests ("Combo") can detect several antigens simultaneously.

During the study, ABON™ PLUS and I-test® RDTs detecting HRP2 and aldolase were used. Among 34 TS-confirmed cases, 67.6% had a positive RDT, and 26.5% had two or more positive tests. RDT sensitivity was 91% in symptomatic patients but only 56.5% in asymptomatic students, likely due to very low parasitemia (<0.1%) below the detection limit (>100 parasites/ μ L). These RDTs are reliable for symptomatic cases with high parasitemia but less effective for screening populations with low parasitemia, such as NPRST (15).

The detection threshold for plasmodial antigens varies by test and species. HRP2, specific to *P. falciparum*, is detectable at approximately 100 parasites/ μ L, whereas aldolase, a pan-specific antigen, shows lower sensitivity for *P. falciparum* and moderate to poor sensitivity for *P. vivax*, *P. ovale*, and *P. malariae* (16,17). In our study, 10

NPRSTs infected with *P. falciparum* were false negative on RDTs, mainly due to low parasitemia, though HRP2 gene deletions or variations may also contribute (18,19). HRP2 expression predominates in asexual and young gametocyte stages, while its homologue HRP3 can cause cross-reactions at high parasitemia. Parasites lacking HRP2 and HRP3 escape detection by HRP2-based RDTs, supporting WHO recommendations to monitor these deletions (1). We noted one case where two positive RDTs enabled diagnosis despite negative microscopy in a patient previously treated with Artemether-Lumefantrine, illustrating that HRP2 persists for several weeks post-treatment, making RDTs useful for diagnosis after treatment but limited for monitoring efficacy (20,21).

During the study, the ABON™ Plus Malaria® test was used on 269 individuals: 23 symptomatic suspected patients and 246 asymptomatic NPRSTs screened systematically. In symptomatic patients, the test demonstrated excellent performance, with a sensitivity of 100%, specificity of 92%, and positive and negative predictive values of 90% and 100%, respectively, consistent with the manufacturer's specifications and previous study findings (22,23). These performances comply with WHO recommendations, which require a minimum sensitivity of 95% for parasitemia ≥ 100 parasites/ μL (24), confirming its usefulness in emergency departments for rapid management decisions. In asymptomatic students, the test's sensitivity dropped to 48%, whereas specificity, positive predictive value, and negative predictive value remained high at 100%, 100%, and 95%, respectively ($\kappa = 0.62$). Consequently, while a positive result is highly reliable, the test carries a risk of false negatives in cases of low parasitemia, which limits its effectiveness for routine screening.

The I-test® test was used in 176 individuals: 10 symptomatic patients and 166 asymptomatic students. According to the manufacturer, the sensitivity for *P. falciparum* is 96.5–100% (threshold 200 parasites/ μL) and for *P. vivax* 90.3–100% (threshold 1500 parasites/ μL), with a specificity of 99.4–100%.

In our study, in symptomatic patients, the test showed 100% sensitivity and excellent agreement with TS ($\kappa = 1$), similar to the ABON™ Plus® test. In contrast, in asymptomatic students, sensitivity was very low (23.8%), with a specificity of 100%, a PPV of 100%, an NPV of 90%, and poor agreement ($\kappa = 0.35$).

As with ABON™ Plus®, I-test® meets WHO requirements for parasitemia ≥ 100 parasites/ μL , but its performance is limited in screening asymptomatic students with low parasitemia. Comparison of the two RDTs shows that, despite the same principle and detection of the same antigens, the source of the antigen and the epitopes targeted by monoclonal antibodies can lead to significant differences in sensitivity and specificity (19).

This study is limited by its retrospective design, leading to incomplete data on travel history, malaria prophylaxis, and patient follow-up. The similar RDT kits used limited direct performance comparisons, and experience with the newly marketed I-test® was insufficient.

Our findings highlight the need to expand malaria

screening to nationals from endemic regions and promote chemoprophylaxis awareness. While RDTs and microscopy have limitations, particularly in asymptomatic students, PCR provides higher sensitivity and accuracy but remains costly and less accessible in our country.

CONCLUSION

Imported malaria remains a public health challenge in Tunisia, mainly affecting travelers and students from sub-Saharan Africa. *P. falciparum* is the predominant species, causing most symptomatic cases with high parasitemia, while asymptomatic infections generally show very low parasite densities that are difficult to detect.

Rapid diagnostic tests, such as ABON™ Plus Malaria® and I-test®, have demonstrated high reliability in symptomatic patients. However, their limited sensitivity in asymptomatic students restricts their usefulness for systematic screening and for patients on chemoprophylaxis. Microscopy remains essential, while PCR represents a promising tool for detecting low parasitemia or cases where conventional diagnosis is challenging.

These findings highlight the importance of targeted screening of at-risk populations, raising traveler awareness about prophylaxis, and strengthening epidemiological surveillance to prevent malaria reintroduction in Tunisia. They also emphasize the limitations of rapid diagnostic tests in imported malaria, informing improved control and surveillance strategies in malaria-free countries.

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