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Field Performance of *Wondfo* and SD Bioline Malaria Pf/Pan Rapid Diagnostic Tests for Malaria Diagnosis in Koraput District, Odisha State, India

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Abstract Rapid diagnostic tests (RDTs) have now been recommended and brought to field operation by the National Vector Borne Disease Control Programme (NVBDCP). The objective of this study was to evaluate the performance of two commonly used RDTs; *Wondfo* and SD Bioline Malaria Pf/Pan for malaria diagnosis in an endemic area of Koraput district, Odisha state. A total of 100 clinically suspected malaria patients at Narayanpatna Community Health Center (CHC) in Koraput district were diagnosed for malaria infection by both microscopy and the two RDTs. Keeping microscopy as the golden standard, sensitivity and specificity of the two RDTs were calculated. Out of the 100 patients recruited for this study, malaria parasites were found in 28 patients by microscopy, of which 42.8%, 17.8%, and 39.3% were *Plasmodium falciparum*, *Plasmodium vivax* and *P. falciparum/P. vivax* mixed infections, respectively. The sensitivity of both *Wondfo* and SD Pf/Pan device was 78.3% and the specificity was 83.1% and 81.8%, respectively for the detection of *P. falciparum.* The sensitivity and specificity of the SD Pf/Pan device for the detection of *P. vivax* was 94.0% and 81.0%, respectively. The *Wondfo* and the SD Bioline Malaria Pf/Pan RDTs performed lower for the diagnosis of *P. falciparum* compared to the gold standard microscopy. Therefore, the accuracy of both the RDTs needs to be improved by enhancing its sensitivity for better management of febrile patients in the endemic situation.

Keywords: *microscopy, plasmodium falciparum, plasmodium vivax, rapid diagnostic tests, Wondfo, SD Bioline Malaria Pf/Pan*

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1. Introduction

Accurate early case detection and complete treatment with appropriate anti-malarial drugs is of prime importance for effective case management among malaria patients. Diagnosis solely based on clinical symptoms is disreputably non-specific and WHO strongly recommends parasitological confirmation of diagnosis by either microscopy or Rapid Diagnostic Tests (RDTs) in all suspected cases of malaria before treatment is started [1]. Despite the wide research in malaria diagnosis, light microscopy-based methods of parasite detection, still remain as the gold standard and the most commonly used one for the diagnosis of malaria parasites in blood samples. However, microscopy also possesses certain limitations such as requirement of well trained microscopist, time consuming, needs careful preparation and application of reagents to ensure quality results etc. RDTs offer a simple and rapid alternative method to microscopic malaria diagnosis, particularly in resource poor endemic settings. Unlike microscopy, RDT results can be obtainable in less than 15-20 minutes which is essential for the appropriate treatment of malaria cases in time.

RDTs are immune-chromatographic test facilitate the diagnosis of malaria by providing evidence for the presence of *Plasmodium*-specific proteins (antigens) such as *Plasmodium falciparum*-specific histidine rich protein 2 (PfHRP2) or *Plasmodium* specific lactate dehydrogenase (pLDH) in human blood with the use of captured monoclonal antibody [2]. Although many products are available in the market, some can detect only one species (e.g. only *P. falciparum*), while others detect further species of the parasite (i.e. *P. vivax*, *P. malariae* and *P. ovale*), in different combinations.

Currently, the use of RDTs for diagnosing *P. falciparum* malaria is being implemented by the National Vector Borne Disease Control Programme (NVBDCP) to improve diagnostic efficiency in peripheral health care settings in India. In Odisha state, the NVBDCP recently introduced one RDT, *Wondfo* which is intended only for the detection of parasite antigen specific to *P. falciparum*. There are different brands of RDT kits used by the private laboratories in India, among which *SD Bioline* Malaria Pf/Pan was the commonly used RDT. The *SD Bioline* Pf/Pan privately used antigen based RDT kit which detects more than one species of malaria parasite indicating differential diagnosis between *P.f* HRP-2 (*P.*

falciparum, histidine-rich protein 2) and other *plasmodium* species (*Pan* LDH). In India, a few studies have evaluated the performance of RDTs in diagnosing malaria and showed varied results in different eco-epidemiological settings [3,4,5]. However, to date, no information is available on the field performance of these two RDTs which are used for malaria diagnosis by the programme or private laboratories in Odisha state. Therefore, we conducted a study to evaluate the diagnostic accuracy of *Wondfo* and *SD Bioline* Pf/Pan RDTs by comparing RDT diagnosis to a high quality microscopy in Koraput, a southern district of Odisha State, which is co-endemic for both *P. falciparum* and *P. vivax [6]*.

2. Methodology

2.1. Study area

The study was conducted during February 2013 to July 2013 in Narayanapatna Community Health Centre (CHC) of Koraput district in Odisha State (Figure 1). Malaria has been endemic in the CHC with two transmission peaks in a year; one during post rainy months (October to November) and the other during summer (March to May) (Source: CDMO office, Koraput). Among the total malaria cases, infection due to *P. falciparum* accounts for >90% in this region. The CHC has recorded the highest Annual Parasite Incidence (API- 123.4) among all the 14 CHCs of the district during 2012 (Source: CDMO office, Koraput). Villages in the CHC situated on hilltops and at the foot of hills are hyper-endemic for malaria with perennial transmission.

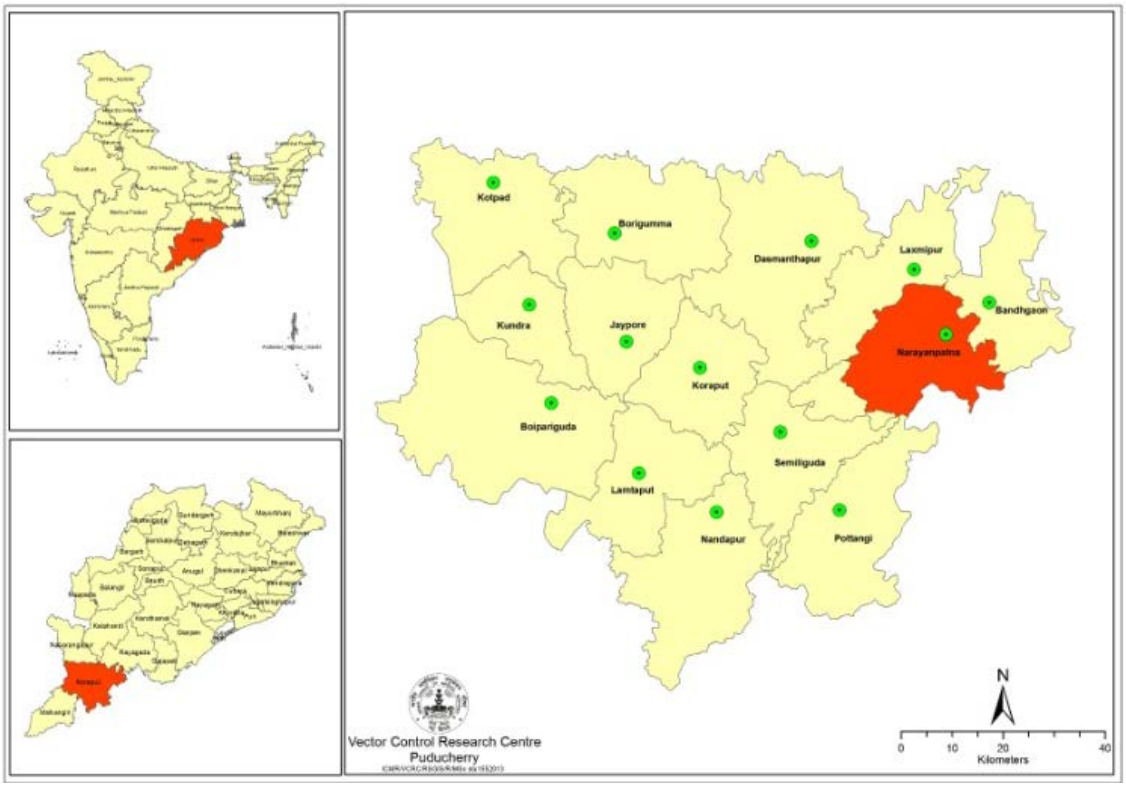

Figure 1. Map showing the study area

2.2. Study Patients and Sample Size

The total sample sizes of 100 patients attending the out-patient section in the CHC were randomly selected for the study based on the symptoms suggestive of malaria. The clinical presumption of malaria was based on febrile illness associated with auxiliary temperature above 37.5°C with or without chills, rigors, sweating, headache and body ache at the time of clinical examination. Patients of any age group and either sex who were considered eligible as clinically presumptive malaria were included in the study. After consultation with the clinician at outdoor section of the CHC, finger prick blood samples were collected by the laboratory technician of the CHC in the presence of a research team member from the suspected patients for the detection of malaria parasites. The patient's profile and his/her clinical data were recorded on a proforma (Patient name, age, sex, village name, history of symptoms etc.,) by the research team member prior to the collection of blood samples. This study was conducted with the request of District health authorities, Koraput.

2.3. Microscopic Examinations

Thick and thin films were prepared by the CHC technician on clean slides with the finger-prick samples following WHO guidelines[7] and handed over them to the research team. After thin films were fixed in methanol and air-dried, slides were stained with 1:10 Giemsa diluted in pH 7.2 buffer and examined by an experienced microscopist of the research team for the presence of malaria parasites at 5x100 magnification with oil immersion lens searching 100 fields (0.25 µl of blood) in each thick smear. Blood parasite density was determined from the thick films [7] by counting the number of asexual parasites per 200 white blood cells and assuming that each individual had 8000 white blood cells/ µl of blood. The slides were classified as negative, if no parasite had been found in 100 oil-immersion fields. To indicate the level of

parasitaemia or parasite density in the positive cases, the number of parasites in thick smears was graded as <100, 100–1000, 1000–10,000, > 10,000 parasites/ µl of blood. As a standard quality control procedure [6], all positive blood smears and 10% of randomly selected negative blood smears were cross-examined by another experienced microscopist of the research team to whom the RDT results were blinded. The results of the second microscopy were combined with that of the first examination.

2.4. Evaluation of Rapid Diagnostic Tests (RDTs) for Malaria

The two RDTs evaluated in the current study were: 1) *Wondfo P.f* (Lot no: W3710908, Mfg. Dt.: 09/09/2011, Expiry Dt.: 08/09/2013, Guangzhou Wondfo Biotech Co. Ltd, Guangdong, China) supplied by the NVBDCP to different CHCs of Odisha State and 2) *SD Bioline* Malaria Pf/Pan (HRP2 and Pan pLDH based) kit (Lot No: N090092, Mfg. Dt.: 17/10/2012, Expiry Dt.: 16/10/2014, Bio-standard diagnostics Pvt. Ltd, Gurgaon, Haryana) procured by the local/private laboratories.

Concurrent with blood smear collection from the fever patients, both the RDTs were performed on the same finger prick blood sample by the laboratory technician of the CHC. The finger prick blood sample was transferred directly to the sample pad using the sample applicator. All RDTs were labeled with patient ID numbers and results were recorded 15 minutes after adding 4 drops (300 µl) of clearing buffer. Appearance of both control and test bands indicated a *P. falciparum/P. vivax infection* and presence of only the control band indicated a negative result. In cases, where the control band did not appear, the results were considered invalid and the tests repeated. Internal quality check included an immediate second reading of all RDTs by a second person of the research team to whom the results of the first reading were blinded. In cases, where there was discordance, the two technicians together re-examined the RDT results and made a collective decision on the reading. Further, the results of the RDTs were blinded to the microscopist who examined the corresponding blood smears of the fever patients.

2.5. Treatment

The patients who were found positive for malaria by RDTs were treated with anti-malarials according to the national guidelines 2013. *P. falciparum* cases were treated with artemisinin combination therapy (ACT) for three days and Primaquine for one day. *P. vivax* cases were treated with chloroquine for three days and Primaquine for 14 days. All mixed infections were treated with full course of ACT and Primaquine for 14 days.

2.6. Data Management and Analysis

Data were entered into case report form and then computerized using Microsoft Excel spreadsheets. The study patients were categorized into four age groups: 1-4, >4-8, >8-14, and >14 years to assess the prevalence of malaria cases by age-group. Comparing with the results of microscopy, sensitivity and specificity of both the RDTs in detecting *P. falciparum* infection were estimated. Several characteristics are used to describe the quality and usefulness of a test. Accuracy is one such characteristic that can be expressed through sensitivity and specificity. Confidence Intervals (CI) (95%) were calculated to reflect the statistical significance of each accuracy measure.

Based on the microscopic results, the RDT results were considered true positive (TP), true negative (TN), false positive (FP), and false negative (FN). Sensitivity, detecting a positive as positive, and specificity, detecting a negative as negative, were calculated as TP/ (TP+FN) and TN/ (TN+FP), respectively. Positive predictive value (PPV) {the proportion of true malaria cases (positive blood smears) among the total number of positive RDTs} and negative predictive value (NPV) {the proportion of true negative malaria cases (negative blood smears) among the total number of negative RDTs} were also calculated. Sensitivity was also stratified by four categories of parasitaemia (<100/ µl, 100–1000/µl, 1000–10,000/µl, > 10,000 parasites/ µl of blood).

3. Results

3.1. General and Clinical Characteristics of Patients

Out of the 100 suspected patients screened for malaria infection using both the RDTs and microscopy, 51 were males and 49 females. The mean age group was 13.5 years ±14 (range 1–60 years old) of which the majority (67%) was below 15 years. The mean duration of fever among the 100 cases was 3.8 ± 0.95 days (range 1–7 days) and their mean body temperature was 38.5 ± 0.72 (range 37.5-40.5°C). Table 1 shows the general and clinical characteristics of the study patients.

Table 1. Patient's Profile With Clinical Data And Malaria Positivity Details

Number of patients tested	100
Mean age (years)	13.5 ±14 (range 1–60 years)
Number of males	51
Number of females	49
Mean Auxiliary body temperature (°C)	38.5±0.72 (range 37.5-40.5)
Mean duration of fever (days)	3.8 ± 0.95 (range 1–7)
Slide positive rate (SPR)	28%
Percentage for only *P. falciparum*	12%
Percentage for only *P. vivax*	5%
Percentage of mixed infection (*P. falciparum* & *P. vivax*)	11%
Mean Parasite density/µl for *P. falciparum* (range)	80-73,800 parasites/µl (mean 14120±16134.9)
Mean parasite density/ µl for *P. vivax* (range)	240-10,300 parasites/µl (mean 1247.5 ±2468.5)

3.2. Microscopy Results

The 100 blood samples obtained from suspected patients were tested for malaria parasites using the two RDTs, *Wondfo Pf*, and *SD Bioline* Malaria Pf/Pan and the results were compared with that of microscopy. Microscopic examination showed that 28% (28/100) of the blood smears were positive for malaria, of which 15 were of males and 13 of females. Prevalence of malaria infections, as detected by microscopy was the highest (78.6%) among subjects aged <14 years indicating that

children were the more susceptible group for malaria infection. Age group wise malaria cases detected by microscopy among the symptomatic patients are illustrated in Table 2.

Table 2. Age Group-Wise Occurrence of Malaria among Symptomatic Patients as Screened by Microscopy

Age group	BSE	Total positive	Positive for *P.f*	Positive for *P.v*	Mixed infection	SPR	SFR	*Pf* %
1-4 yrs	30	9	1	3	5	30.0	20.0	66.7
>4-8 yrs	25	8	5	0	3	32.0	32.0	100.0
>8-14 yrs	12	5	2	0	3	41.7	41.7	100.0
>14 yrs	33	6	4	2	0	18.2	12.1	66.7
Total	**100**	**28**	**12**	**5**	**11**	**28.0**	**23.0**	**82.1**

BSE- Blood slide examined
SPR- Slide positivity rate
SFR- Slide *falciparum* rate
***Pf* %-** *Plasmodium falciparum* percentage.

Among the 28 malaria-positive patients, 12 patients (42.8%) were infected only with *P. falciparum*, 5 patients (17.8%) only with *P. vivax* and 11 patients (39.3%) had mixed infections with *P. falciparum* and *P. vivax*. Thus, in total, 23 *P. falciparum* cases and 16 *P. vivax* cases were found positive which include the mixed infections. The parasite density by microscopy ranged from 80-73,800 parasites/µl of blood (mean 16184.7 ± 16233.2) for *P. falciparum* and from 240-10,300 parasites/µl of blood (mean 1247.5 ± 2468.5) for *P. vivax*.

3.3. Wondfo RDT Results for *P. falciparum*

Microscopically confirmed *P. falciparum* cases were 23, of which *Wondfo* RDT kits detected 18 matching positives. There were five cases found as FN and 13 as FP by *Wondfo* RDT. The sensitivity and specificity of the RDT for *P. falciparum* were 78.3% (95% CI, 61.4-95.1) and 83.1% (95% CI, 74.1-91.5) and the PPV and NPV were 58.1% (95% CI, 40.7-75.4) and 92.8% (95% CI, 86.6-98.9), respectively. False-positive and false-negative rates were 0.17 and 0.22 respectively. The true HRP-2 test yielded an overall accuracy of 82% (95% CI, 74.5-89.5). Overall, performance characteristics of RDTs compared with gold standard microscopy are given in Table 3. Two subjects of positive *P. falciparum* with low parasitaemia (80 and 120 parasites/µl of blood) and one subject with a parasitaemia of 1800 parasites/µl of blood were tested negative by this RDT. But, still this RDT has detected one case of positive for *P. falciparum* at the density of 80 parasites/µl of blood. Two subject's positive with high parasite density (13760 & 13200 parasites/µl) for *P. falciparum* were detected negatives by the RDT. When a parasitaemia of more than 100 parasites/µl of blood was considered, the tests were 81.0% sensitive for the detection of *P. falciparum*, which was lesser than the criteria set by the WHO. The test showed a sensitivity of only 50% in detecting *P. falciparum* when the parasitaemia was below 100 parasites/µl of blood. The sensitivity of the RDTs in relation to the degree of parasitaemia for the detection of *P. falciparum* is shown in Table 4.

Table 3. Field Performance of the RDTs Compared with Microscopy

Test Characteristics	*Wondfo Pf*	SD Bioline Pf/Pan (for *P. falciparum)*	SD Bioline Pf/Pan (for *P. vivax)*
True positives	18	18	15
False positives	13	14	16
True negatives	64	63	68
False negatives	5	5	1
Sensitivity % (95% CI)	78.3 (61.4-95.1)	78.3 (61.4-95.1)	93.8 (81.9-100)
Specificity % (95% CI)	83.1 (74.1-91.5)	81.8 (73.2-90.4)	81.0 (72.6-89.3)
Positive predictive value (PPV) % (95% CI)	58.1 (40.7-75.4)	56.3 (39.1-73.4)	48.4 (30.8-66.0)
Negative predictive value (NPV) % (95% CI)	92.8 (86.6-98.9)	92.6 (86.4-98.9)	98.6 (95.7-100)
False positive rate	0.17	0.18	0.19
False negative rate	0.22	0.22	0.06
Accuracy %	82 (74-90)	81 (73-90)	83 (76-90)

C.I- Confidence intervals.

Table 4. Sensitivity of the Rapid Diagnostic Tests by Parasite Density (Microscopy

Parasites/µl of blood	No. positive by microscopy	*Wondfo P.f* & SD Bioline Pf/Pan		SD Bioline Pf/Pan	
		For *P. falciparum*		For *P. vivax*	
		No. positive	Sensitivity (%) 95% CI	No. positive	Sensitivity (%) 95% CI
<100	2	1	50% (8.2-91.8)	0	0.0
100–1000	2	1	50% (8.2-91.8)	11	91.7(61.4-98.6)
1000–10,000	6	5	83.3 (36.1-97.2)	3	100(30.5-100)
> 10,000	13	11	84.6 (54.6-97.6)	1	100(16.5-100)

3.4. SD Bioline Malaria Pf/Pan Result for *P. falciparum*

In total, 18 out of the 23 *P. falciparum* positive cases (as detected by microscopy) were correctly identified by the *SD Bioline* Malaria Pf/Pan RDT and five cases were shown as FN. A total of 63 microscopy confirmed negative samples (TN) and 14 samples were found as FP. The sensitivity and specificity of the *SD Bioline* Malaria Pf/Pan RDT for the detection of *P. falciparum* were 78.3% (95% CI, 61.4-95.1) and 81.8% (95% CI, 73.2-90.4) and the PPV and NPV were 56.3% (95% CI, 39.1-73.4) and 92.6% (95% CI, 86.4-98.9), respectively. False-positive and false-negative rates were respectively 0.18 and 0.22. Overall accuracy of the SD RDT was 81% (95% CI, 73.3-88.7) (Table 3).

Out of the 5 FN detected by this RDT, two subjects were with low parasitaemia (80 and 120 parasites/µl of blood) and one with moderate parasitaemia of 1800 parasites/µl of blood. However, the RDT detected one case of positive for *P. falciparum* at a density of 80 parasites/µl of blood. Two subject's positive with high parasite density (13760 & 13200 parasites/µl of blood) for *P. falciparum* were tested negative for malaria by the RDT. When a parasitaemia of more than 100 parasites/µl of blood was considered, the tests were 81.0% sensitive for the detection of *P. falciparum*, which was lesser than the criteria set by the WHO. The test showed a sensitivity of 50% for detecting the *P. falciparum* when the parasitaemia was below 100 parasites/µl of blood (Table 4).

3.5. SD Bioline RDT Results for Non Falciparum Species

SD Bioline Malaria Pf/Pan RDT detects other *Plasmodium* species (*P. vivax*, *P. ovale*, and *P. malariae*) pLDH antigen in the blood. But in the current study, as no positives for *P. malariae* and *P. ovale* parasites were detected, appearance of 'Pan' line was presumably considered positive for *P. vivax*. The *SD* test detected 15 out of the 16 microscopically confirmed *P. vivax* cases. There was one subject found as FN by the test which had a parasitaemia of 320 parasites/µl of blood. Of the 68 microscopically confirmed negative samples (TN) and 16 samples were found as FP. Total of 5 *P. falciparum* positive samples had cross reacted with 'Pan' line (pLDH) and produced a FP line for *P. vivax*. The sensitivity and specificity of the test were 93.8% (95% CI, 81.9-100) and 81% (95% CI, 72.6-89.3) respectively in detecting *P. vivax* compared to the sensitivity of the test in detecting *P. falciparum* infections (78.3%), the sensitivity of detecting *P. vivax* cases was much higher. The positive and negative predictive value of the test was 48.4% (95% CI, 30.8-66.0) and 99% (95% CI, 95.7-100) respectively, (Table 3) with the false-positive and false-negative rates of 0.19 and 0.06 which were lesser compared to the rates for the detection of *P. falciparum*. Overall accuracy of *SD Bioline* Malaria Pf/Pan RDT for the detection of *P. vivax* was 83% (95% CI, 75.6-90.4).

The test showed a sensitivity of 93.7% in detecting the *P. vivax* infections when the parasitaemia was more than 100 parasites/µl of blood and no positive case was found for *P. vivax* with below 100 parasites/µl of blood (Table 4).

4. Discussion

RDTs play an essential role in the current malaria control programme particularly in rural inaccessible endemic areas of India. In Odisha state, under the NVBDCP, RDTs have been used for diagnosis of malaria (only for *Pf*) at peripheral level since 2005. Initially, Para Hit-*f*, RDT kit was used in the malaria control programme for detection of HRP-2 specific to *P. falciparum. Wondfo* which is also intended for detecting HRP-2 of *P. falciparum* has been used subsequently since 2010 by the NVBDCP in endemic malarious areas of the State, which are inaccessible or where microscopic facilities are either poor or lacking. Apart from this, the private laboratories in the State commonly use *SD Bioline* Malaria Pf/Pan RDT kit. In the current study, the diagnostic accuracy of the NVBDCP supplied monovalent *Wondfo* and privately used SD Bioline Malaria Pf/Pan RDT were evaluated relative to microscopy.

It is expected that an appropriate malaria RDT should have high sensitivity (95%) and specificity (95%) and ability to detect at least 100 malaria parasites/µl of blood as the set criteria by WHO [8]. In the current study, the sensitivity and specificity of the RDTs used for the detection of *P. falciparum* showed lower performance than the criteria set by the WHO. The results also showed a lower performance of the two RDTs in detecting *P. falciparum* infections compared to the previously tested RDT kits in India which had a sensitivity of 90-96% and specificity of 88-95% for *P. falciparum*[3-4].

The sensitivity and specificity of SD Bioline Malaria Pf/Pan RDT were found inconsistent when compared to other studies where it performed with better accuracy and showed a sensitivity range of 90.2 to 93.6% and a specificity of 81.2-98.5% [9-11]. But, when compared to the results of a study conducted in Nigeria, the performance of the RDT accuracy was lower in the diagnosis of *P. falciparum*, with a sensitivity and specificity of 75.2 and 80.4% respectively [12]. Similarly, in the past, only two studies assessed the Wondfo RDT for diagnosis of P. falciparum and showed highly satisfactory results with a sensitivity of 95.5% and 88.6% [13,14]. The published field trials of RDTs showed high variability in their performance due to several factors including difference in the ecological settings as reported elsewhere [8].

The First Response Malaria Combo (pLDH/HRP2) card tested in an earlier study in the forested belt of central India showed 83% sensitivity and 94% specificity for *P. vivax* [5]. In the same area, another study with ICT *Pf/Pv* showed 72% sensitivity for *P. vivax*[3]. However, in the current study, *SD Bioline* Malaria Pf/Pan RDT performed superior (93.8% sensitivity) in the diagnosis of *P. vivax* compared to the results the earlier two studies in central India [3,5]. The current study finding is corroborate with the two previous reports which assessed the SD Bioline Malaria Pf/Pan RDT for P. vivax in both endemic and non-endemic settings outside India, where it performed well with the sensitivity of 96.4 % and 88% respectively [15,16].

Factors that affect the sensitivity and the specificity of RDTs are the formidable challenges for malaria diagnosis particularly detection of parasite at low parasitaemia. A 95% sensitivity at 100 malaria parasites/µl of blood has been recommended as a target for RDT performance by

the WHO[8]. In the current study, when the parasitaemia was more than 100 parasites/µl of blood, both the antigen based tests were 81% sensitive for the detection of *P. falciparum*, which is lesser than the WHO recommended threshold of 95%. The tests were only 50% sensitive for detecting *P. falciparum* when the parasitaemia was below 100 parasites/µl of blood. Thus, the results of the current study clearly showed that both the RDTs did not meet the WHO performance criteria. However, in the current study, there was no scope of calculating sensitivity of the RDTs in relation to the parasite density less than 100 parasites/µl of blood, since there were only two *P. falciparum* cases with such low level of parasite density and there was no *P. vivax* case under this category of parasitaemia. It has been suggested that lower levels of HRP2 during a malaria episode with low parasitaemia might not be detected by RDTs [17] because malaria antigenaemia depends on the parasite load in the patient's body during an acute episode [18].

Under good conditions, RDTs should achieve a low level of false-negatives, similar to levels commonly achieved by microscopy. This is important from the clinical perspective because false-negative results due to under diagnosis can lead to failure to treat a potentially fatal disease. In the present study, there were also 5% false-negative results (*P. falciparum)*, besides the better performance. Moreover, the major clinical concern was the failure of the rapid tests in detecting parasites even when they were in high densities leading to false negative results as recorded earlier [4]. In the current study, both the RDTs failed to detect two positive cases out of the five false negatives for *P. falciparum* with the parasite density of 13760 and 13200 parasites/µl of blood. Other workers have similarly observed false negative results in some patients with high parasitaemia, up to 18,000/µl of blood [19]. The limitation of the present study is generalization of the results based on less sample size. Hence, it could not be concluded precisely the sensitivity of the RDTs based on parasite density.

An important criterion for the selection of RDTs in the endemic setting is the ability to detect other human malaria species in addition to *P. falciparum*. The current study area has been endemic for both *P. falciparum* and *P. vivax* malaria. Mixed infections of these two parasite species are common in the area. *P. falciparum* and *P. vivax* infections require different drug regimen. Misdiagnosis of mixed species infections as single species infections would lead to improper drug treatments where the *P. vivax* parasite has a tendency to form the relapse. Hence, *Pf*/ *Pv* RDT device is highly necessary for the current study area. But, the ongoing malaria control programme in Odisha state use the monovalent RDT (*Wondfo*) which diagnose only *P. falciparum* infection and needs to be changed to bivalent RDT. Further studies need to be conducted to explain the possible reasons for false negative RDT results at high density of parasiteamia in both *P. falciparum* and *P. vivax* cases.

Narayanapatna CHC is an inaccessible area with a vast hilly terrain. Majority of the people are tribes. From the analysis of epidemiological data available in the CHC, it was understood that malaria transmission was high in the study area as supported by the current study that reported a Slide Positivity Rate (SPR) of 28%. From malaria transmission control perspective, the RDTs can play a key role in this CHC in rapid diagnosis and complete treatment of malaria. There is every possibility for an inordinate delay in examining the blood smears by one malaria laboratory technician positioned in CHC Head quarters. The delay in getting the results of microscopic diagnosis is a serious obstacle for malaria control in remote areas where health staffs have to visit several times to treat the positive cases as per the results of microscopic examination. Hence, use of RDT is a best option in this scenario with the prevalence of both the malaria parasites round the year. Taking the upper hand in the existing situation, the private practioners play an important role in providing health facilities to the people living in remote areas. Most of them use *SD Bioline* Malaria Pf/Pan RDT for diagnosing malaria and since the sensitivity of this RDT was only 78.3% for detecting *P. falciparum*, this needs to be improved.

5. Conclusion

The *Wondfo* and *SD Bioline* RDTs performed with lower accuracy for the diagnosis of *P. falciparum* compared to the gold standard microscopy. Therefore, the accuracy of both the RDTs needs to be improved by enhancing its sensitivity for better management of febrile patients in the endemic situation. Further, correct diagnosis of mixed infections should be an important criterion for the selection of a RDT to be used in the programme. Hence, the RDT which detects both *P. falciparum* and *P. vivax* parasite is highly necessary for the current study area.

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Conflict of Interest Statement

The authors declare that they have no conflict of interests.

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