SENSITIVITY AND SPECIFICITY OF TWO HRP-2/PLDH COMBINATION RAPID DIAGNOSTIC TEST FOR THE DETECTION OF PERIPHERAL MALARIA IN PREGNANT WOMEN IN SOUTH WEST SUMBA, INDONESIA

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ABSTRACT

Introduction and Objectives: Malaria in pregnancy remains a big problem in the world, whether in areas with stable transmission or unstable transmission. Few studies have been done to evaluate the sensitivity and specificity of RDT as a screening tool of malaria in pregnant women. But there is still lack of data from low transmission areas such as Indonesia. The objective of this study was to evaluate the sensitivity and specificity of two different HRP2/pLDH combination RDT for detecting peripheral malaria in pregnant women.

Methods: A cross-sectional study was conducted in a period of six weeks, from mid May until end of June 2011. Enrolment was conducted at one Hospital and two Puskesmas in South West Sumba District.

Results: The accuracy of First Response Malaria Ag Combo against PCR was better than Carestart™ Malaria HRP-2/pLDH for detecting P. falciparum malaria in peripheral blood of pregnant women (sensitivity 57.14% vs 33.33%, specificity 99.09% vs 99.08%, respectively). In detecting PAN plasmodium, the sensitivity was 20% for both RDT. The specificity was 99.54% and 99.55, for First Response Malaria Ag Combo and Carestart™ Malaria HRP-2/pLDH, respectively.

Conclusion: First Response was better for the detection of P. falciparum from peripheral blood of pregnant women. Both RDT showed a low sensitivity for detecting peripheral PAN plasmodium infection in pregnant women. However, low number of positive cases which were behind the sample size needed to be accounted before making any recommendation.

Keyword: RDT, sensitivity and specificity, malaria in pregnancy, sumba
INTRODUCTION
Malaria in pregnancy is a major public health problem. Dellicour et al (2010) estimated a 54.7 million pregnancies at risk of malaria in stable transmission of *Plasmodium falciparum*, and 70.5 million other in areas with very low malaria transmission.

The prevalence of malaria in pregnancy varies between areas, from 10% to as high as 65%, with up to 200,000 infant deaths due to malaria infection during pregnancies. (Steketee et al, 2001). Recent estimation by World Health Organization (WHO) quantified that in endemic areas in Africa, there are 30 million pregnancies at risk of malaria infection. (Guyatt et al, 2004).

In Indonesia, around 70 million people get malaria each year, where Papua and Sumba are provinces with the highest prevalence. (Statistics Indonesia et al, 2007). High rate of malaria infection of *P. falciparum* and *Plasmodium vivax* in pregnant women may cause maternal anaemia, preterm birth and low birth weight (Poespoprodjo et al, 2008).

Many studies have evaluated the performance of rapid diagnostic test (RDT) in detecting malaria in various areas in general population. Only few studies have evaluated the sensitivity and specificity of RDT for the screening of malaria in pregnancy (VanderJagt et al 2005; Tagbor et al 2008). Most of the studies were conducted in Africa where transmission is mainly by *P.falciparum*. Studies in low transmission areas and in areas where both *P.falciparum* and *P.vivax* coexisting particularly in pregnant women are limited. Thus this study is one of the few where HRP-2 and parasite Lactate Dehydrogenase (pLDH) combination RDTs are being evaluated for screening pregnant women in Indonesia in a low transmission region of *P. falciparum* and *P. vivax*.

The effect of *P. falciparum* and *P. vivax* for the clinical burden of malaria in pregnancy is already known. However, the role of RDT in detecting *P. vivax* in pregnant women is limited. Hence, RDTs with the ability to detect both species in pregnant women is needed. This study will evaluate the use of HRP2/pLDH combination rapid diagnostic test which can detect *P. falciparum* and pan *plasmodium* in Indonesia where both species exist. Result of this study will be benefit for the implementation of malaria in pregnancy control program using RDT.

METHODS
Study Site: The study was conducted in South West Sumba District, Indonesia. Samples were enrolled from the referral hospital of the district, Karitas Hospital, and two community Health centers.

Study Design: A cross sectional study was conducted in a period of six weeks, started from mid May until end of June 2011.

Sample Size: The sample size was calculated based on expected sensitivity and specificity, minimum acceptable lower confidence limit, and a 12% prevalence of the disease based on standard guidelines found in Flahault et al (2005) for evaluating accuracy of diagnostic test. The minimum sensitivity recommended by WHO for a diagnostic test is 95%. With an expected sensitivity of 0.98 and lower 95% confidence limit > 0.85 with 0.95 probability, the minimum number of positive RDTs required is 50. To get this positive cases, based on the disease prevalence of 0.12, and the formula: $N_{controls} = N_{cases} \times \frac{(1-Prev)}{Prev}$, the number of negative RDTs required is 365, so the study need to enrol at least a total of 415 women from antenatal clinics and delivery units.

Enrolment: All pregnant women who came to the hospital or community health centers for routine antenatal care (ANC) was screened for eligibility and offered to join the study. Eligibility criteria were: (1) 15-49 years old, (2) Able to give informed consent, (3) Have no known allergy to the antimalaria DHP. Participant were excluded when they were pregnant women with symptoms of severe malaria or any other condition of concomitant infections requiring hospital admission and pregnant women with mental disability that prevents informed consent

Study Procedures: Prior to the study enrolment, and short refreshment training were given to the midwives regarding questionnaire filling, blood sample collection, and result forms filling. The microscopists were also being trained for microscopy result forms filling.

All pregnant women who were willing to participate in the study were asked to sign the informed consent. Followed by registration and questionnaire filling by the midwives. Subsequently, blood sample
collection was done by a finger prick. The blood sample was screened for malaria by RDTs microscopy examination and PCR.

Rapid Diagnostic Test
The study used two different malaria RDTs. Carestart™ Malaria HRP-2/pLDH (Pf/pan) Combo Test (Access Bio Inc, New Jersey, USA) and First Response Malaria Ag Combo (HRP-2/pLDH) (Premier Medical Corporation Ltd, India) was chosen based on a recent evaluation by WHO/TDR and FIND of RDT products. Carestart™ Malaria HRP-2/pLDH (Pf/pan) Combo Test (Access Bio, New Jersey, USA) was the best for the detection of Plasmodium vivax, and First Response Malaria Ag Combo (HRP-2/pLDH) (Premier Medical Corporation Ltd, India) was the best for Plasmodium falciparum (WHO 2010b).

Each test was labelled with patient’s ID and date before testing, and was carried out according to manufacturer’s instruction. Results were recorded as species, species, or invalid. Tests with invalid result did not being repeated. All the RDTs were kept at a room temperature recommended by the manufacturer at the study site and opened just before performing the test.

Microscopy
Microscopy reading was done twice. First reading was done by field microscopist and second reading was done by expert microscopist which was blinded to the first microscopy reading result. Both microscopist were blinded to the RDT results. Microscopy ready result was categorized as negative, positive, undetermined, or cannot be read.

PCR
For PCR analysis, filter papers were used to collect the blood samples. The PCR procedure was conducted in Eijkman Institute for Molecular Biology Jakarta. The laboratory technician who performed this procedure was masked from the RDT and microscopy results.

Data Analysis: Epidata Entry (Version 3.1) was used to enter the data, and then transferred to PASW Statistics 18 for analysis. To calculate the sensitivity and specificity of RDT and microscopy, results were classified as true positive, true negative, false positive and false negative. Results then being summarized in the standard 2x2 table and the sensitivity, specificity, positive and negative predictive values were calculated with degree of uncertainty determined by 95% confidence interval. The RDT is the index test and expert microscopy and PCR as the interim gold standard. PCR was also used for discrepant resolution of all RDT and microscopy positive and negative results.

Ethical Approval: The study was reviewed and approved by the Research Ethics Committee Liverpool School of Tropical Medicine, and the Eijkman Institute for Molecular Biology, Jakarta, Indonesia. In addition, informed consent was obtained from each participants.

RESULTS
Based on PCR findings, the prevalence of malaria in the study was 1.47%. A total of 10 positive cases was found, with 6, 3, and 1 cases of P. falciparum, P. vivax, and mixed infection between P. falciparum and P. vivax, respectively.

Baseline Characteristic of Study Population
A total of 681 participants were included in the analysis. The biggest number of participant came from group age of 25-29 years (n=204, 30%), followed by group age of 30-34 years and 20-24 year (n=178, 26.1% and n=164, 24.1%, respectively). Most of the participants live in rural areas (98.5%) with Kori, Mangganipi and Ate Dalo were the villages with the highest participants (12%, 7.3%, and 7.% respectively). Seventy nine percent of the participants (n=540) have been living in the area for more than 10 years, and the rest are 10 years or below.

Literacy was still a problem in the area, where nearly half of the population could not read (48.9%). The ones who were able to read mostly finished elementary school (n=174, 25.6%). Only 1.3% women graduated from university.

Number of ANC visit was various from 1 to 9, with a mean of 2.82. From 681 participants, 15.6% (n=106) were primigravida and the rest was multigravida, with a mean of 3.01. The highest number of previous pregnancy from the participants was 11 (0.6%) which was found in 3 women from group age of 40-44 years old, and 1 woman from group age of 30-34 years old. Over 90% participants never had a dead born child nor abortion/miscarriage (91.9% and 93.8%, respectively), but a total of 4 dead born child
was found in 2 (0.3%) participants, and 1 (0.1%) participant experienced 5 times abortion/misscarriage.

There were 44.1% (n=300) participants reported own a bed net, where 97% of them slept under the net the night before. Of these participants, 83.67% (n=251) reported that they slept under the bednet everyday. There were 32.9% of all participants (n=224) own a treated bednet, and 26.0%(n=177) knew that the bed net was treated again the year before. Only 2.5% (n=17) of all participants took a preventive therapy for malaria in pregnancy. But there was 2.2% (n = 15) of the participants took some medicines but did not know whether it was malaria preventive drugs or not. The drugs taken for preventive therapy was chloroquine, paracetamol and a combination of the two of it (3.03%, 21.21%, and 18.18%), and 57.6% of participant did not know what kind of drugs they received. Only 6 participants reported the source of preventive drugs the consumed, which was from antenatal care clinic in the community health centres (n=4), Karitas health service house (n=1), and Puskesmas Pembantu (Pustu) (n=1).

Sensitivity, Specificity, and Predictive Values
The accuracy of RDT and microscopy was measured against PCR as the gold standard. The calculation was differentiated for P. falciparum and PAN plasmodium.

The sensitivity for Carestart™ Malaria HRP-2/pLDH (Pf/pan) Combo Test against PCR for P. falciparum was 33.33%, and the specificity was 99.08%. The positive predictive value was 25% and negative predictive value was 99.39%.

Table 1. Carestart™ Malaria HRP-2/pLDH (Pf/pan) Combo Test (Access Bio Inc, New Jersey, USA) against PCR for P. falciparum

<table>
<thead>
<tr>
<th>PCR</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDT Carestart</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>2</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>649</td>
<td>653</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>655</td>
<td>661</td>
</tr>
</tbody>
</table>

For First Response Malaria Ag Combo (HRP-2/pLDH), the sensitivity against PCR for P. falciparum was higher than Carestart™ Malaria HRP-2/pLDH (Pf/pan) Combo Test, which was 57.14%. But for the specificity it has a similar number, which was 99.09%. Positive predictive value and negative predictive value were 40% and 99.55%, respectively.

Table 2. First Response Malaria Ag Combo (HRP-2/pLDH) against PCR for P. falciparum

<table>
<thead>
<tr>
<th>PCR</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDT First Response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>660</td>
<td>663</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>666</td>
<td>673</td>
</tr>
</tbody>
</table>

Sensitivity for detecting P. falciparum by Entebe was 0%, given the positive predictive value 0% too. However, the specificity was 99.28%, and the negative predictive value was 98.34%.

Table 3. Field microscopy against PCR for P. falciparum

<table>
<thead>
<tr>
<th>PCR</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field Microscopy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>641</td>
<td>645</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>644</td>
<td>651</td>
</tr>
</tbody>
</table>
For expert microscopy, the sensitivity for detecting *P. falciparum* against PCR was 42.86%, whereas the specificity was 100%. The positive predictive value was 100% and the negative predictive value was 99.41%.

### Table 4. Expert microscopy against PCR for *P. falciparum*

<table>
<thead>
<tr>
<th>PCR</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expert Microscopy</td>
<td>Positive</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>4</td>
<td>670</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>7</td>
<td>670</td>
</tr>
</tbody>
</table>

Carestart™ Malaria HRP-2/pLDH (Pf/pan) Combo Test showed a low sensitivity against PCR in detecting PAN plasmodium, which was 20%. However, the specificity was 99.54%. Positive predictive value and negative predictive value for Carestart™ Malaria HRP-2/pLDH (Pf/pan) Combo Test were 40% and 98.78%, respectively.

### Table 5. Carestart™ Malaria HRP-2/pLDH (Pf/pan) Combo Test (Access Bio Inc, New Jersey, USA) against PCR for PAN plasmodium

<table>
<thead>
<tr>
<th>PCR</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDT Carestart</td>
<td>Positive</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>8</td>
<td>648</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>10</td>
<td>651</td>
</tr>
</tbody>
</table>

Not so different with the previous RDT, First Response Malaria Ag Combo (HRP-2/pLDH) also showed a low sensitivity of 20% and low positive predictive value of 40% in detecting PAN plasmodium against PCR. The specificity was 99.55% and the negative predictive value was 98.80%.

### Table 6. First Response Malaria Ag Combo (HRP-2/pLDH) against PCR for PAN plasmodium

<table>
<thead>
<tr>
<th>PCR</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDT First Response</td>
<td>Positive</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>8</td>
<td>660</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>10</td>
<td>663</td>
</tr>
</tbody>
</table>

Similar with the result showed in detecting *P. falciparum*, the sensitivity of Entebie in detecting PAN plasmodium was also 0%, brings the positive predictive value also 0%. The specificity was 99.27% and negative predictive value was 97.62%.

### Table 7. Field microscopy against PCR for *P. vivax*

<table>
<thead>
<tr>
<th>PCR</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field microscopy</td>
<td>Positive</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>4</td>
<td>629</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>4</td>
<td>649</td>
</tr>
</tbody>
</table>

The accuracy of expert microscopy showed 75% sensitivity against PCR for detecting *P. vivax* in peripheral blood of pregnant women. Furthermore, the specificity was 99.41%, positive predictive value was 42.86%, and negative predictive value was 99.85%.
Table 8. Expert microscopy against PCR for *P. vivax*

<table>
<thead>
<tr>
<th>Expert microscopy</th>
<th>PCR Positive</th>
<th>PCR Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>670</td>
<td>671</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>674</td>
<td>678</td>
</tr>
</tbody>
</table>

**DISCUSSION**

**Prevalence**

The prevalence of malaria in pregnancy found in the study (1.47%) was far lower than what was found by the previous study by Syafruddin et al (personal communication). This might due to the short rainy season in the previous months before the study conducted. Another ongoing research about vector control conducted in the surrounding areas of the research also found difficulties in catching *Anopheles* mosquito as the vector of malaria.

**Baseline Characteristics**

Categorizing the age into group age was a proper decision, since most of the women in Sumba did not know about their date of born. With most of the participant did not speak Indonesian language, a direct communication between researcher and participant became limited. The role of midwives who could speak the local language in the study was very important for the communication.

With a range from 1 to 9, and a mean of 2.82 for the number of ANC, showed that the ANC program in the village has started to run effectively and reached the community. This is possibly because the ANC service was not only run at the Puskesmas, but also there were schedules of ANC services where the midwives will go to in each villages and give free examination, hence the mothers did not have to go to the Puskesmas.

By the time the study conducted, the distribution of free insecticide treated bednets to pregnant women was still in the beginning phase. That was why the number of pregnant women who own a bednet was not even half. Considering the economic reason, it was less likely for mothers to spent some money for buying a bednet.

What was lack from the distribution of bednets by the staff of the Puskesmas were no information about the content and maintenance of the bednet. Many of the women who already had a bednet did not know that the bednet needed to be treated each year, especially after being washed for several times.

Preventive therapy for malaria in pregnant women was not a common practice found in Puskesmas in Sumba. Not like other places in Africa, the recommendation for malaria preventive therapy in Indonesia still does not exist.

**Accuracy of the test**

In this study, RDT First Response Malaria Ag Combo (HRP-2/pLDH) showed the highest number of sensitivity for detecting peripheral *P. falciparum*, and expert microscopy showed the best sensitivity for the detection of peripheral *P. vivax* in pregnant women. None of the RDT showed a good sensitivity in detecting *P. falciparum* (33.33% and 57.14% for Carestart™ Malaria HRP-2/pLDH (Pf/pan) Combo Test and First Response Malaria Ag Combo (HRP-2/pLDH), respectively), nor in detecting *P. vivax* malaria in pregnant women (20% and 20% sensitivity of *Carestart™ Malaria HRP-2/pLDH (Pf/pan) Combo Test and First Response Malaria Ag Combo (HRP-2/pLDH))

In the other hand, specificity of all test for *P. falciparum* showed a good number of above 99%. A similar result also found for the detection of *P. vivax*, except for field microscopy which had a sensitivity of below 99% (96.92%).

However, it is important to remember that considering the limited number of sample size where the positive cases did not met the expected number, the results could not be said representative.

Using PCR as the gold standard in this study was an advantage since it is known to have a more sensitive and specific for diagnosis than microscopy (Ashley et al 2009). However, this method is not practical to be applied in the field where there are limited resources. Moreover, not like microscopy, PCR could not give the
information about parasite stage and the number of parasitaemia. A careless PCR technique also can affect the results of the PCR.

In this study, expert microscopy showed a good specificity in detecting *P. falciparum* and *P. vivax* of 100% and 99.41%, respectively. This result is conformable with the study from Snounou (2007) which expected to have a specificity of expert microscopy against PCR of close to 100%.

**Study procedures**

Questionnaire filling were found to be difficult by the midwives. A lot of jump questions were ignored by the midwives. This might be happen because of the short period of training time in filling questionnaires.

RDT was supposed to be a simple, practical, and easy to perform tool to help health workers in diagnosing malaria. In this study, the recruitment was did by researcher and the midwives, hence the midwives needed to do the test. Most midwives, even the researcher, found some difficulties in performing two RDTs at the same time, along with producing the blood smear and collecting blood spot on filter papers. Nevertheless, it got better the more often they did it.

Shortage of staff was also a problem in the study site, especially at Karitas Hospital. With lack of staff and the abundant workload, it was difficult to do the recruitment without delaying the staff primary job. At the other study sites, Puskesmas Kori and Puskesmas Bondo Kodi, the midwives set up a special ANC day together with the recruitment for the study for each village, hence the workloads from the Puskesmas were not interfered.

Difficulties in reading the blood smear was experienced by the microscopist, either field microscopist or expert microscopist. The problem arose because of the low quality of malaria blood smears made by the midwives. The midwives do not normally make a blood smear for malaria. Even though some of the midwives have been trained before on how to make a blood smear, a half day refreshment training on how to make a malaria blood smear was not sufficient for the to regain the capability. Especially when they have not been doing it for months.

**CONCLUSION**

**Main Findings**

1. The prevalence of peripheral malaria in pregnancy in South West Sumba area from mid May until end of June 2011 was 1.47%. The sensitivity and specificity of Carestart™ Malaria HRP-2/pLDH (Pf/pan) Combo Test against PCR for the detection of *P. falciparum* were 37.33% and 99.08%, respectively.

2. The sensitivity and specificity of First Response Malaria Ag Combo (HRP-2/pLDH) against PCR for the detection of *P. falciparum* were 57.14% and 99.09%, respectively.

3. For the detection of PAN plasmodium against PCR, Carestart™ Malaria HRP-2/pLDH (Pf/pan) Combo Test and First Response Malaria Ag Combo (HRP-2/pLDH), showed a similar number of 20% for the sensitivity and 99.5% for the specificity.

4. The numbers presented above are much lower compare with similar studies conducted earlier. A small number of positive cases must be taken into consideration for the results found in this study, hence the non representative point of the number.

5. Based on the study, First Response Malaria Ag Combo (HRP-2/pLDH) was a better choice for the detection of *P. falciparum* malaria. No RDT can be recommended for the detection of pan plasmodium malaria, based on the findings.

6. There is a need of regular refreshment training for field microscopist to maintain their reading skill.

**Recommendations for further research**

1. A longer period of time for collecting samples is needed to accommodate enough sample size.

2. Shortage of staff at the study site can be managed by putting an additional research staff to do the enrolment.

3. A shorter and simplified questionnaire should be generated to avoid the confusion for the midwives. Training on how to fill in the questionnaire in a more
thorough and practical way, such as doing a role play, might be an option. Using a PDA for entering the questionnaire data was also an option, though this option is not economically friendly.

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