

**RESEARCH ARTICLE**

**Comparison between the Diagnostic Performances of Rapid Diagnostic Test (RDT) using Advantage Malaria Card Pf/Pv Ag, Microscopy, and Polymerase Chain Reaction (PCR) in Malaria Suspected patients at the Merauke Regional General Hospital**

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**ABSTRACT:**

Malaria prevalence in Indonesia is still high, especially in eastern Indonesia, namely Papua, reaching a peak of 16%, for example, in Merauke. The diagnosis of Malaria is conducted according to the gold standard, using the microscopy method. However, it is still limited, raising the need for a Rapid Diagnostic Test (RDT) examination. This study aims to evaluate the diagnostic performances of Immunochromatography (ICT) based RDT (using Advantage Malaria Card Pf/Pv Ag), compared to the diagnostic performances of microscopy and Polymerase Chain Reaction (PCR). This cross-sectional observational study took all fever patients of outpatient and inpatient care at the Merauke Regional General Hospital (hereinafter referred to as RSUD Merauke) from June to July 2019 as the samples. The sample population included all malaria patients with positive microscopy results, and the control population included all non-malarial fever patients at RSUD Merauke. Each specimen underwent microscopy (thick and thin preparations), RDT, and Real-Time (RT)-PCR tests using a Rotor Gene-Q (Qiagen) with abTES™ malaria 5qPCR III reagent. The diagnostic performances of RDT were analyzed by calculating its sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV). The study was conducted on 105 samples, namely 55 malaria samples and 50 control samples, who underwent microscopy, RDT, and RT-PCR. RDT sensitivity to microscopy, *P. vivax* 100%, *P. falciparum* 89.5%; specificity of *P. vivax* 95.7%, *P. falciparum* 97.7%. RDT sensitivity to RT-PCR, *P. vivax* 53.5%, *P. falciparum* 48.6%; specificity of *P. vivax* 100%, *P. falciparum* 98.5%. The RDT p-value for microscopy and RT-PCR was p=0.000. RDT k-coefficient of microscopy, *P. vivax* 0.937, *P. falciparum* 0.871. RDT k-coefficient of PCR, *P. vivax* 0.427, *P. falciparum* 0.531. RDT is more sensitive to *P. vivax* than microscopy. RDT is more specific to *P. vivax* than RT-PCR. Further studies are suggested to discuss anti-malaria drug resistance and sequencing.

**KEYWORDS:** Malaria, Diagnosis, Microscopy, RDT, Real-Time PCR.

**INTRODUCTION:**

Malaria is one of the diseases that has been discovered since Greek times. This disease is quite typical and easily recognizable by a regularly recurrent fever accompanied by chills.

The terms febris tertiana and febris quartana have been used at that time. Malaria is often found in swampy areas emitting a foul smell around it. It is the basis for naming 'malaria', which is composed of two syllables, 'mal' (bad) and 'area' (air), so it can be interpreted as bad air<sup>1</sup>.

namely *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi*<sup>2,3,4</sup>.

Based on the world malaria report, it was estimated that in 2010 there were 219 million cases of malaria with 660,000 death cases. In 2011, it was estimated that from 104 endemic countries, 80% of deaths due to malaria occurred in only 14 countries, and 80% of cases occurred in 17 countries. In 2010, there were 22.9% new cases with a malaria prevalence of 10.6% in Indonesia, predominantly in eastern Indonesia, especially in rural areas classified as remote areas<sup>3</sup>.

Approximately 16% of Papua's population is at risk of being infected with malaria. The clinical malaria rate in Papua is recorded at 198 per 1,000 population. It is estimated that the number of clinical malaria patients is far above the recorded number, considering that the *puskemas* (public health centre) reporting system is not routinely conducted so that in 2000, the clinical malaria morbidity rate reached 210,991 cases or 101.16 per 1,000 population<sup>1,5</sup>.

Malaria data at the Merauke Regional General Hospital (hereinafter referred to as RSUD Merauke) in 2015 revealed 396 malaria cases, consisting of 141 cases of *Plasmodium falciparum* malaria and 255 cases of *Plasmodium vivax* malaria. In 2016, it increased to 411 cases, consisting of 206 cases of *Plasmodium falciparum* malaria and 205 cases of *Plasmodium vivax* malaria. The 2017 data displayed 373 malaria cases, consisting of 179 cases of *Plasmodium falciparum* malaria and 170 cases of *Plasmodium vivax* malaria<sup>6</sup>. The 2018 data of Merauke District Health Affairs Office indicated 4,850 malaria cases, consisting of 1085 cases of *Plasmodium falciparum* malaria, 3703 cases of *Plasmodium vivax* malaria, 3 cases of *Plasmodium ovale* malaria, 3 cases of *Plasmodium malariae* malaria, no cases of *Plasmodium knowlesi* malaria, and 56 cases of mixed *Plasmodium sp*<sup>7</sup>.

A malaria parasite test examines the blood of patients suspected of being infected with malaria, either by a microscopic test or a Rapid Diagnostic Test (RDT). A patient can be declared positive for malaria when the microscopy (thick and thin preparations) indicates *Plasmodium sp* in the blood or when the RDT demonstrates a positive result. Many techniques have been developed to detect early transmission of malaria, from clinical, serological (RDT, ELISA), to molecular (e.g. Polymerase Chain Reaction, PCR) tests<sup>1-7</sup>.

This study aims to evaluate the diagnostic performances of the immunochromatography (ICT) based rapid diagnostic test (RDT) using advantage malaria card

Pf/Pv compared to diagnostic performances of the gold standard test (microscopy) and confirmatory PCR.

**METHOD:**

It is a cross-sectional observational study, taking all fever patients of outpatient and inpatient care at RSUD Merauke from June to July 2019 as the samples. The sample population included all malaria patients with positive microscopy results, and the control population included all non-malarial fever patients at RSUD Merauke. Each specimen underwent microscopy (thick and thin preparations), RDT, and Real-Time (RT)-PCR tests using a Rotor Gene-Q (Qiagen) with abTES™ malaria 5qPCR III reagent. Inclusion criteria were all patients suspected of being infected with malaria, both male and female, from all age groups, having an excellent supportive clinical examination, and willing to participate in the study by signing informed consent. The exclusion criteria were patients with lysed blood samples and those unwilling to sign the informed consent. The immunochromatographic diagnostic performances of the Advantage malaria card were analyzed by calculating the diagnostic sensitivity, diagnostic specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV). The suitability test between immunochromatography, microscopy, and Real-Time PCR tests was determined by *Cohen's Kappa Coefficient* (κ). The analysis was conducted with Microsoft Excel and SPSS version 25.

**RESULTS:**

**Characteristics of Malaria and Control Samples**

Table 1 shows the study conducted on 59 malaria samples (with four samples excluded), so the total was reduced to 55 samples and 50 control samples (non-malaria).

**Table 1. Characteristics of Malaria and Control Samples**

Characteristics	Malaria (N= 55)	Control (N=50)
Sex		
a. Male	36	28
b. Female	24	22
Age (Year)		
a. 0-4 year	1	2
b. 5-14 year	5	14
c. 15-24 year	13	7
d. 25-34 year	15	4
e. 35-44 year	12	7
f. 45-54 year	6	7
g. ≥ 55 year	5	9
Malaria Microscopy		
a. <i>P. falciparum</i>	19	0
b. <i>P. vivax</i>	34	0
c. <i>P. ovale</i>	0	0
d. <i>P. malariae</i>	0	0
e. Mix	2	0

**Table 2. Comparison Between the Sensitivity and the Specificity of RDT and Microscopy**

Types of Plasmodium	Microscopy (N=105)		Sensi (%)	Speci (%)	PPV (%)	NPV (%)	p-value*	Kappa coefficient
	Positive	Negative						
RDT <i>P.v</i>			100	95.7	92.1	100	0.000	0.935
a. Positive	35	3						
b. Negative	0	67						
RDT <i>P.f</i>			89.5	97.7	89.5	97.7	0.000	0.871
a. Positive	17	2						
b. Negative	2	84						

Descriptions: malaria RDT (Advantage Pf/Pv Ag), Pv (*Plasmodium vivax*), Pf (*Plasmodium falciparum*), Sensi (Sensitivity), Speci (Specificity), PPV (positive predictive value), NPV (negative predictive value), \*(p< 0.0001)

**Table 3. Comparison Between the Sensitivity and the Specificity of RDT and Real-Time PCR**

Types of Plasmodium	Real-Time PCR (N=105)		Sensi (%)	Speci (%)	PPV (%)	NPV (%)	*p-value	Kappa coefficient
	Positive	Negative						
RDT <i>P.v</i>			53.5	100	100	50.7	0.000	0.427
a. Positive	38	0						
b. Negative	33	34						
RDT <i>P.f</i>			89.5	97.7	89.5	97.7	0.000	0.871
a. Positive	17	2						
b. Negative	2	84						

Descriptions: malaria RDT (Advantage malaria), Pv (*Plasmodium vivax*), Pf (*Plasmodium falciparum*), Sensi (Sensitivity), Speci (Specificity), PPV (positive predictive value), NPV (negative predictive value), \*(p< 0.0001)

**Comparison between the Sensitivity and Specificity of Rapid Diagnosis Test (RDT) and Microscopy:**

Table 2 shows the comparison between the sensitivity and the specificity of RDT and microscopy, with a sensitivity of 100% and a specificity of 95.7% for *P. vivax*, and a sensitivity of 89.5%, and a specificity of 97.7% for *P. falciparum*.

**Comparison Between the Sensitivity and the Specificity of RDT and Real-Time PCR**

Table 3 displays the comparison between the sensitivity and the specificity of RDT and Real-Time PCR, with a sensitivity of 100% and a specificity of 95.7% for *P. vivax*, and a sensitivity of 89.5%, and a specificity of 97.7% for *P. falciparum*.

**DISCUSSION:**

Table 1 indicated that male patients were more dominantly infected with malaria than females, which was in line with a study by Basuki et al. (2009), Hanandita W, and Tampubolon (2016). However, it contradicted the results of a study by Gunawan (2000), indicating that the prevalence difference according to age and sex was influenced by differences in the level of immunity due to variations in exposure to mosquito bites<sup>11</sup>. *P. vivax* was found to have a more dominant frequency than other species, according to research by Fransisca L et al. (2015). However, Bourgeois et al. (2010) and Berzosa P et al. (2018) discovered that *P. falciparum* was more dominant, potentially resulting from the differences in the distribution and the number of samples<sup>8-15</sup>.

Table 2 displayed the comparison between the sensitivity of RDT and microscopy, with higher sensitivity for *P. vivax* and higher specificity for *P.*

*falciparum*. Sharma J et al. (2013) performed microscopic examinations on *P. falciparum* and *P. vivax*, obtaining a sensitivity of 81.25% and a specificity of 95.83%, and on the RDT Advantage malaria card, obtaining a sensitivity of 91.67% and a specificity of 85.42%. However, this study did not specify the type of Plasmodium<sup>14</sup>.

Microscopic examination with Giemsa stain is still the gold standard for malaria diagnosis. However, the results can be considered reliable only if performed by an experienced expert. The drawback is the subjectivity of the examiner, especially in diagnosing mixed infections or infections with low parasite counts. In addition, in an advanced stage of *P. falciparum* infection occurring in the internal capillaries (sequestration), the parasites are difficult to observe in the peripheral blood, thus requiring serial blood examinations (3 times in 48 hours) to confirm the presence or absence of the parasites<sup>9</sup>.

Various studies have shown a reasonably high sensitivity and specificity compared to microscopy as the gold standard. The sensitivity of RDT with HRP-2 in diagnosing *P. falciparum* can reach 95%. Meanwhile, for diagnosing *P. vivax* infection, the sensitivity of pLDH-based RTD is frequently lower than 90%, and the aldolase-based RTD has even lower sensitivity, approximately 40%. In addition, the number of parasites (especially at the asexual stage) also affects the RDT results; the higher the number of parasites, the more likely the test can be positive and vice versa (unreliable). Several studies reported that HRP-2 could persist in the blood of treated patients for up to 28 days, although the microscopic examination indicated negative

parasitaemia. In contrast, evaluation of treatment using the LDH enzyme revealed that the RDT results were negative within seven days of treatment<sup>1-3,9,16,17,18</sup>.

Table 3 demonstrated the comparison between the sensitivity of RDT and real-time PCR, with higher sensitivity for *P. vivax* and higher specificity for *P. falciparum*. It was supported by research by Berzosa et al. (2018)<sup>13</sup>.

The nucleic acid-based parasite diagnosis was conducted using reporter DNA molecules to detect specific DNA or RNA sequences belonging to a particular parasite. The parasite specimen, which was the target of the diagnosis, was lysed by damaging the parasite membrane in various ways, such as using alkaline solutions, detergents, heat, urea, guanidine, or sound waves to remove and denature the nucleic acid. Molecules that can serve as reporters include oligonucleotides, DNA fragments, single-stranded RNA, or plasmid DNA<sup>1,19,20,21,22,23,24</sup>.

The use of the PCR test depends on its sensitivity and specificity. In asymptomatic patients, the PCR test can be applied to measure treatment success. The possibility of cross-reaction with other organisms present in a population needs to be considered since it can influence the predictive value of the test. PCR test is the most sensitive method in detecting malaria parasites in the blood, and the sensitivity can reach five parasites/ $\mu$ L; some even report up to one parasite/ $\mu$ L. In addition, PCR has a more reliable specificity in diagnosing the species of Plasmodium. In several reports, a single malaria infection based on microscopy and RDT turned out to be a mixed infection after PCR testing. With the nested or multiplex PCR techniques, the results can only be seen in 1-2 days, while with the real-time PCR technique, the results can be seen within 2 hours. However, PCR diagnosis is not yet widely applicable in Indonesia because it is costly and requires sophisticated equipment and special training<sup>1,2,9,25,26,27</sup>.

## CONCLUSIONS:

Malaria is one of the diseases that has been discovered since Greek times<sup>1</sup>. Malaria is caused by the Plasmodium parasite (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*) transmitted through the bite of the female Anopheles mosquitoes<sup>1-129</sup>. This cross-sectional observational study took all fever patients of outpatient and inpatient care at RSUD Merauke from June to July 2019 as the samples. The sample population included all malaria patients with positive microscopy results, and the control population included all non-malarial fever patients at RSUD Merauke. Each specimen underwent microscopy, RDT, and Real-Time RT-PCR tests. The diagnostic performances of the RDT using Advantage malaria card were analyzed by

calculating the diagnostic sensitivity, specificity, PPV, and NPV. The suitability test between RTD, microscopy, and RT-PCR tests was determined by Cohen's Kappa Coefficient ( $\kappa$ ).

This study was conducted on 55 malaria samples and 50 control samples. Male patients were more dominantly infected with malaria than females. The prevalence difference according to age and sex was influenced by differences in the level of immunity due to variations in exposure to mosquito bites<sup>11</sup>. *P. vivax* was found to have a more dominant frequency than other species, according to a study in 2015. However, other studies suggested that *P. falciparum* was more dominant, which potentially resulted from the differences in the distribution and the number of samples<sup>11-13,15</sup>.

The comparison between the sensitivity and the specificity of RDT and microscopy indicated a sensitivity of 100% and a specificity of 95.7% for *P. vivax*, and a sensitivity of 89.5%, and a specificity of 97.7% for *P. falciparum*. Sharma J et al. (2013) performed microscopic examinations on *P. falciparum* and *P. vivax*, obtaining a sensitivity of 81.25% and a specificity of 95.83%, and on the RDT Advantage malaria card, obtaining a sensitivity of 91.67% and a specificity of 85.42%<sup>14</sup>. Microscopic examination with Giemsa stain is still the gold standard for malaria diagnosis. However, the results can be considered reliable only if it is performed by an experienced expert, and still, it is greatly influenced by the subjectivity of the examiner. The number of parasites also affects the RDT results. HRP-2 could persist in the blood of treated patients for up to 28 days, while the LDH enzyme could be negative within seven days of treatment<sup>1-3,9</sup>. The comparison between the sensitivity and the specificity of RDT and RT-PCR indicated a sensitivity of 100% and a specificity of 89.5% for *P. vivax*, and a sensitivity of 89.5%, and a specificity of 97.7% for *P. falciparum*<sup>13</sup>. PCR test is the most sensitive method in detecting malaria parasites in the blood, and the sensitivity can reach one to five parasites/ $\mu$ L. In addition, PCR has a more reliable specificity in diagnosing the species of Plasmodium. However, PCR diagnosis is not yet widely applicable in Indonesia because it is costly and requires sophisticated equipment and special training<sup>1,2,9</sup>. Further studies are suggested to discuss anti-malarial drug resistance and sequencing.

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