ISSN 2089 - 2136

Tropical Medicine Journal

Volume 03, No. 2, 2013

TMJ	Volume 03	Number 02	Page 95 - 195	ISSN 2089 - 2136

Center for Tropical Medicine, Faculty of Medicine, Universitas Gadjah Mada
in collaboration with Indonesian Society of Tropical Medicine and Infectious Disease (PETRI)

Address : Tropical Medicine Journal, PAU Building, Jalan Teknika Utara Barek, Yogyakarta
Universitas Gadjah Mada, Yogyakarta 55281, Phone : +62-274-588483, E-mail: tropmedjournal@gmail.com

TROPICAL MEDICINE JOURNAL

ISSN : 2089-2136
Center for Tropical Medicine, Faculty of Medicine, Universitas Gadjah Mada in collaboration with Indonesian Society of Tropical Medicine and Infectious Disease (PETRI)

Volume 03, Number 02

CONTENTS

Validity of p-LDH/HRP2-Based Rapid Diagnostic Test for the Diagnosis of Malaria on Pregnant Women in Maluku

Vebiyanti[1], E. Elsa Herdiana Murhandarwati[2,3], Bambang Udji Jokorianto[3]
[1]Faculty of Medicine, Universitas Pattimura, Maluku Tengah, Indonesia; [2]Department of Parasitology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia; [3]Center for Tropical Medicine, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Corresponding author: vebi_tentua@yahoo.com

ABSTRACT

Introduction: Pregnant women are one of the groups at risk for infection by the malaria parasites in endemic areas. The dangerous impacts of malaria in pregnancy are anemia and severe malaria that can cause death for mother, fetus and newborn. Clinical symptoms that are likely to be not typical until asymptomatic in pregnancy are one of the obstacles on diagnosing malaria in pregnancy in endemic areas. p-LDH/HRP2-RDT (*Pf/Pan)* is one of the WHO recommended RDT product on round 1-4 and has been used in Maluku. This tool is able to detect antigens of the *Plasmodium* metabolism results in peripheral blood so that it is regarded to be more sensitive than microscopic examination. The use of p-LDH and HRP2-RDT (*Pf/Pan*) for the detection of *P. falciparum* HRP-2 antigen and *P. vivax, P.malariae, P.ovale* p-LDH antigen have not been previously evaluated in the Province of Maluku.

Objectives: To evaluate the validity of p-LDH/HRP2-RDT (*Pf/Pan)* compared with microscopic examination and nested Polymerase Chain Reaction (PCR) as the gold standard for the diagnosis of malaria in pregnancy in Maluku.

Methods: This was a cross-sectional study using a diagnostic test of malaria in pregnant women. The study was conducted in Ambon City health center, Savana Jaya Buru Island health center and Haulussy Ambon Local Hospital. Sample data, the data of pregnancy, RDT results and microscopic results on the field were recorded in the questionnaire. Nested PCR examination was conducted at the Laboratory of Parasitology, Faculty of Medicine, Universitas Gadjah Mada as well as second reading for microscopic examination

Results: The results showed that p-LDH/HRP2-RDT (*Pf/Pan)* had the same sensitivity with micoscopic of 11%, a specificity of 100% higher than microscopic 96% compared with nested PCR as the gold standard, p-LDH/HRP2-RDT (*Pf/Pan)* had PPV and NPV of 100% and 98% compared with nested PCR as the gold standard. p-LDH/HRP2-RDT (*Pf/Pan)* sensitivity was 80% compared to the microscopic examination.

Conclusion: diagnostic malaria in pregnancy in Maluku with p-LDH/HRP2-RDT (*Pf/Pan)* was less sensitive than nested PCR and microscopic.

Keywords: Malaria, pregnant woman, diagnostic test, validity, p-LDH/HRP2 *Rapid Diagnostic Test* (RDT) (*Pf/Pan)*

INTISARI

Pendahuluan: Wanita hamil adalah salah satu kelompok berisiko malaria di daerah endemis. Dampak malaria pada kehamilan seperti anemia dan malaria parah dapat menyebabkan kematian bagi ibu, janin dan bayi baru lahir. Gejala klinis yang mungkin tidak khas sampai asimtomatik pada kehamilan adalah salah satu kendala dalam mendiagnosis malaria dalam kehamilan di daerah endemik. RDT p-LDH/HRP2 Pf/Pan adalah salah satu rekomendasi WHO pada putaran 1-4 dan telah digunakan di Maluku. Alat ini mampu mendeteksi antigen dari hasil metabolisme *Plasmodium* dalam darah perifer sehingga dianggap lebih sensitif dibandingkan mikroskop. Penggunaan p-LDH/HRP2 Pf/Pan untuk mendeteksi *P. falciparum* HRP-2 antigen dan p LDH dari *P. vivax*, *P.malariae*, *P.ovale* antigen belum pernah dievaluasi di Propinsi Maluku.

Tujuan: penelitian ini adalah untuk mengevaluasi validitas p-LDH/HRP2 Pf/Pan dibandingkan dengan pemeriksaan mikroskopis dan *nested Polymerase Chain Reaction* (PCR) sebagai standar baku untuk diagnosis malaria dalam kehamilan di Maluku.

Metode: Penelitian ini merupakan studi cross-sectional menggunakan tes diagnostik malaria pada wanita hamil. Penelitian dilakukan di Kota Ambon Puskesmas, Puskesmas Savana Jaya Pulau Buru dan Haulussy RSUD Ambon. Data sampel, data kehamilan, hasil RDT dan hasil mikroskopis di lapangan dicatat dalam kuesioner. Pemeriksaan *Nested PCR* dan mikroskopis (*cross-check*) dilakukan di Laboratorium Parasitologi, Fakultas Kedokteran, Universitas Gadjah Mada.

Hasil: Validitas RDT p-LDH/HRP2 Pf/Pan memiliki sensitivitas sama dengan mikroskopik yaitu 11%, spesifisitas 100% lebih tinggi dibandingkan spesifisitas mikroskopik yaitu 96% dengan *nested PCR* sebagai standar baku emas. p-LDH/HRP2-RDT (*Pf/Pan)* memiliki nilai PPV dan NPV yaitu 100% dan 98% dibandingkan *nested PCR* sebagai standar baku emas. Sensitivitas p-LDH/HRP2-RDT (*Pf/Pan)* adalah 80% dibandingkan dengan pemeriksaan malaria dengan mikroskopis.

Simpulan: diagnosis malaria menggunakan p-LDH/HRP2-RDT (*Pf/Pan)* pada ibu hamil di Provinsi Maluku sebagai daerah endemis malaria, memiliki nilai sensitivitas yang lebih rendah dibandingkan dengan pemeriksaan nested PCR dan mikroskopis.

Kata kunci: Malaria, wanita hamil, tes diagnostik, validitas, RDT p-LDH/HRP2 (*Pf/Pan)*

INTRODUCTION

Until currently, malaria has been one of the infectious diseases that causes death in the world after tuberculosis[1]. One of the risk groups infected with malaria is pregnant women. The impact of malaria in pregnant women is anemia, low birth weight and abortion; therefore, this is a threat to malaria endemic areas. The number of pregnant women and infants who die due to malaria and severe anemia due to malaria is 10,000 pregnant women and 200,000 babies each year[1,2]. Cases of malaria especially in pregnant women are still a problem in Eastern Indonesia, including Maluku. Maluku is one of provinces with the highest API (Annual Parasite Incidence) in Eastern Indonesia[3].

One of the WHO strategies for malaria control in pregnant women is the early diagnosis of malaria in pregnant women with microscopic examination and the use of RDTs, thus appropriate malaria therapy could prevent the effects of malaria in pregnancy for both mother

and fetus[4]. Although malaria examination with the thick blood preparation can detect the number of parasites 50-100 parasites/µl, the sensitivity is dependent on several factors e.g. microscopic examination of slide-making skills, microscopic reading accuracy or availability of proper equipment[5]. RDT offers some conveniences such as easier to use in the field since it does not require special skills and provides faster results. Regarding the sensitivity, RDT could detect >100 parasites/ul in the peripheral blood[6]. However, RDT sensitivity is also influenced by various factors, namely: parasitic factors (species and degree of parasitemia, parasite antigen structure variability and antigen ability); factor of RDT condition, technical factors of the use of RDT, and its interpretation[7].

A low number of parasites in the peripheral blood is due to sequestration process in the placenta by *P. falciparum* infection because the attachment of VAR2CSA protein antigen on the surface of erythrocytes with Chondroitin Sulfate A (CSA) and Hyaluronic acid (HA) receptors from the placenta is produced in the second trimester[8.9.10]. This causes microscopic examination to be less sensitive than RDT which is able to detect antigens as the metabolism results of *Plasmodium* in blood. On infection by *P. vivax*, the sequestration process in the placenta does not happen, but *P. vivax* infection during pregnancy is likely to lead to babies born with low birth weight[8].

Degree of parasitemia is also influenced by the immunity system. Pregnant women who are infected with malaria and living in endemic areas will have the ability to suppress parasitemia and develop protective specific immunoglobulins (IgG) and cell mediated immunity (anti-parasite immunity) so that the symptoms of malaria in pregnant women become oligosymptomatis to asymptomatis on very low parasitic conditions in the blood due to antitoxic immunity[11,12].

p-LDH/HRP2-RDT Pf/Pan is one of RDT products recommended by the WHO in round 1 to round 4 for use in zone 2, like Asia that has a mixed *P. falciparum* infection of *P. vivax* and *P. malariae* as well as *P. ovale*[13]. This tool has the form of cassette containing membrane strip covered by a specific monoclonal antibody against HRP-2 at the Pf line for the detection of *P. falciparum* and specific polyclonal antibodies against p-LDH at the Pan lines for detection of *P. vivax, P. ovale and P. malariae*[13,14]. Antigen of p-LDH on HRP2-RDTPf/Pan has the lower sensitivity than HRP-2 antigen. The sensitivity for the detection of *P.vivax* is only 50% compared to the microscopic as the standard, if the number of parasites is 1-50 parasites/µl of blood, and the sensitivity for the detection of P*. falciparum* from 93.8 % up to 100 % compared to the microscopic as the standard, if the number of parasites is 1-50 parasites/ µl of blood[14].

PCR is a method to amplify DNA fragments in a short period of time[15]. This test is very specific and sensitive (approaching 100 %). This test can detect at least 2 parasites, even 1 parasite/µl blood[16,17], but this PCR test has disadvantage: a) the provision of DNA and RNA primer is very complicated, b) the tools used for hybridization are complex, c) tools for PCR amplification and detection of amplification product is very sophisticated and expensive, d) it takes a long time (24 hours) so that it is only suitable for epidemiological and experimental studies[16].

This study is to evaluate the validity of p-LDH/HRP2-RDT (*Pf/Pan)* compared with microscopic examination and nested Polymerase Chain Reaction (PCR) as the gold standard for the diagnosis of malaria in pregnancy in Maluku.

MATERIALS AND METHODS

In this cross sectional study, the population was pregnant women in Ambon City Public Health Center (PHC), Haulussy Ambon Local Hospital and Savana Jaya P. Buru Health Center. The inclusion criteria included pregnant women who lived and settled in the Maluku; pregnant women with symptoms of fever (temperature > 37.4 °C) in the past 2 weeks; pregnant women without symptoms of fever, but had any of the following sign: anemic conjunctiva, weakness, fatigue, lethargy, muscle pain, headache, vaginal bleeding, nausea and vomiting, and diarrhea. The exclusion criteria included pregnant women who were taking antimalarial drugs or taking malaria drugs within the last 2 weeks and pregnant women who were not willing to sign the informed consent of research. Fifty nine pregnant women in this study who met the inclusion and exclusion criteria were recruited.

The variables assessed in this study were: sensitivity, specificity, positive predictive value, negative predictive value of p-LDH/HRP2-RDT (*Pf/Pan)* tool compared with microscopic. Nested PCR is used as the gold standard. Informed consent had been signed by all who participated in the study. Permit of the study was approved by the research ethics committee of the Faculty of Medicine, Gadjah Mada University. Tests done were:

a. Microscopic examination (developing thick and thin blood smear preparation)

Malaria thick and thin slides were prepared, stained and counted according to WHO (2011) in Basic Malaria Microscopy 2nd edition[4].

b. Examination with Rapid Diagnostic Test

p-LDH/HRP2-RDT was provided in the form of cassette. RDT examination was used to assess the presence of HRP-2 antigen in infections (*P. falciparum*) and p-LDH antigen in *P. vivax, P.malariae, P. ovale* infections. The RDT examination was done by two health personal (midwifes or nurses). Before examining RDT, RDT officers ensured that it was not expired. Blood specimens taken with a disposable loop were inserted into the rounded-shape sample well. The officers then put four drops of assay buffer into the box-shaped assay diluent hole next to the sample well and allowed it to stand for fifteen minutes and read. Results should not be read after 30 minutes. Result was considered as positive Pf (*P. falciparum*) when 2 lines (test line "Pf" and the control line "C") were appeared in the result window; a positive Pan (*P. vivax, P.malariae, P. ovale*) if 2 lines (test line "Pan" and the control line "C") were appeared on the window; mixed infections (*P. falciparum and P. vivax or P.malariae, P. ovale*) if 3 lines (test line "Pf", test line "Pan" and control line "C") were appeared on the window. Result was considered as negative if only one control line "C" was appeared in the result window. No color that appeared on the control strip means that the examination should be repeated[14].

c. Nested PCR examination

This examination was performed in the laboratory of Parasitology of FM-UGM, Yogyakarta. Peripheral blood samples were collected on filter paper (Whatman 3 MM chromatography paper). DNA was then extracted from the filter paper with a method and isolated using Chelex method.

Nested PCR was done through two steps, for nested one, a pair of primer was used rPLU 1 and rPLU 5. Second amplification was nested two for genus (rPLU 3 and 4) followed by nested two for species (rFAL 1 and rFAL2, rMAL1 and rMAL2, rVIV1 and rVIV2, rOVA1 and rPLU2 primers[21]. PCR condition was set as follow: initial denaturation 94^{0}C for 1 minute, annealing 59^{0}C for 2 minutes, extension 72^{0}C for 5 minutes, denaturation repetition 94^{0}C for 1 minute annealing 59^{0}C for 2 minutes, extension 72^{0}C for 5 minutes and repeated 35 times.Nested PCR was done as suggested by CR product for nested two genus was positive at 240 bp, and nested species was confirmed positive when a band with specific base pairs was appeared (205 bp for *P. falciparum*, 144 bp for *P.malariae*, 117 bp for *P. vivax*, and 226 bp for *P. ovale*[21].

Validity of the diagnostic tool of p-LDH/HRP2-RDT (*Pf/Pan)* including the sensitivity, specificity, positive predictive value, negative predictive value was then assessed by comparing the RDT data with microscopy and nested PCR as the gold standard.

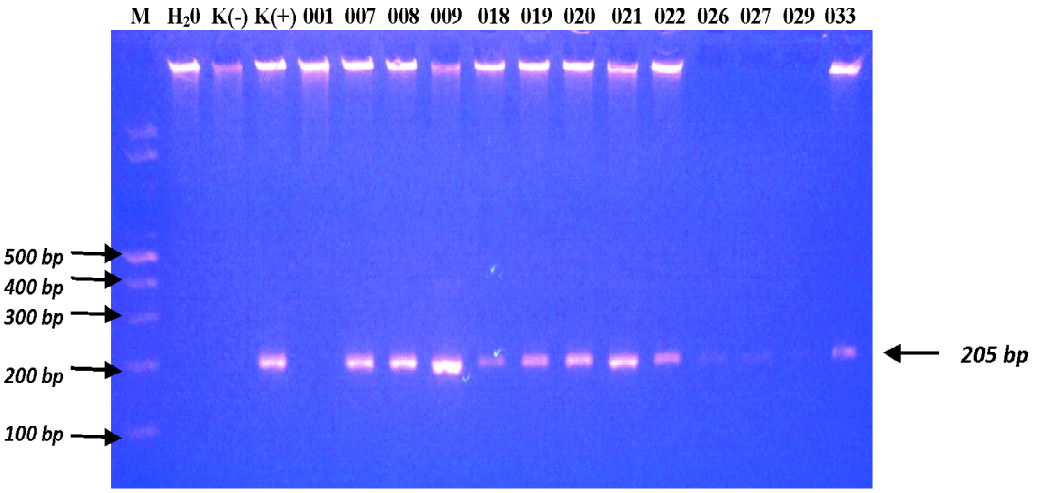

Figure 1. Example of Nested 2 PCR spesific spesies result showed the presence of *P. falciparum* of blood samples taken from pregnant wowen at Ambon Primary Health Center, Savana Jaya Primary Health Center, Haulussy General Hospital, Ambon on September 2012 – April 2013

RESULTS AND DISCUSSION

In this study, the prevalence of malaria in pregnant women with RDT Pf / Pan is 6.8% (4/59), a microscopic 8.5% (5/59), 61% (36/59) nested PCR. Maternal age with the highest prevalence of malaria using PCR is in 21-29 age group by 27 (67.5%). The prevalence of malaria in pregnant women in first pregnancy and two pregnancy as much as 23/39 (58.9%) less than multigravida malaria infection in 13/20 (65%) by PCR, in contrast to previous studies that found the prevalence of malaria is more common in the first and second pregnancies compared to multigravida[23].

The risk of reinfection in pregnant women in malaria-endemic areas will cause the immune system has the ability to suppress parasitemia[11,12]. A low number of parasites in the blood due to suppression of the immune system and the effects of sequestration of the malaria parasite of *P. falciparum* can cause the sensitivity of the RDT is become less[7,19]. The low number of parasites in the blood, requires malaria RDTs as diagnostic tool with the high sensitivity in malaria endemic areas.

Table 1. Comparison of the results of p-LDH/HRP2 RDT *P.f/Pan,* and *nested* PCR of pregnant women in September 2012 – April 2013 in Ambon Primary Health Center; Savana Jaya (P.Buru) Primary Health Center and Haulussy General Hospital in Ambon City.

RDT *P.f/Pan*		*Nested* PCR		Total
		Positif	Negatif	
	Positif	4	0	4
	Negatif	32	23	55
Total		36	23	59

The results of the validity of RDT Pf /Pan (samples of peripheral blood) in this study for the examination of malaria in pregnant women have a sensitivity of 9.75%, specificity 100%, PPV 100%, NPV 42% when compared with nested PCR as standard gold standard (Table 1). Meanwhile, RDT sensitivity SD Pf/Pan compared to microscopic examination was 80%, 100% specificity, 100% PPV and 98% NPV (Table 2). The sensitivity results of RDT Pf / Pan in this research is lower than the sensitivity of the RDT Pf / Pv 83.3% in Eastern Sudan. The difference of the sensitivity of the RDT tool can be influenced by differences in the criteria of the samples taken and the status of malaria in different areas[18].

Table 2. Comparison of the results of p-LDH/HRP2 RDT *P.f/Pan,* and microscopic of pregnant women in September 2012 – April 2013 in Ambon Primary Health Center; Savana Jaya (P.Buru) Primary Health Center and Haulussy General Hospital in Ambon City.

		Microscopic		Total
		Positif	Negatif	
RDT *P.f/Pan*	Positif	4	0	4
	Negatif	1	54	55
Total		5	54	59

RDT sensitivity 80%, specificity 100%, PPV 100%, NPV 98% compared to the microscopic. In contrast to the results of research in Sei Barombang, Labuan Batu, North Sumatra found that Parascreen RDT test results had a sensitivity of 0% and a specificity of 100% compared with the gold standard microscopic.[24] This difference may be due in Sei Barombang samples were taken from all pregnant women (with and without symptoms of malaria), whereas in this study the samples taken from pregnant women with malaria symptoms who are typical and not typical, so it can provide higher sensitivity results.

In this study, factors that could affect the validity of RDT are the condition of RDT, the use of RDT and interpretation techniques[7] of p-LDH/ HRP2-RDT Pf/Pan has been controlled by the researchers with keeping the RDT in the right temperature at 4^0C-30^0C and use it before the expired periods of RDT. The researchers disseminate and training on how to use the RDT tool to clinic staff and hospital personnel before the study so that officers can use RDTs correctly and can read it right interpretation of the results of the RDT.

The degree of parasitemia that can be seen by the microscope showed that the type of *P. vivax* malaria is highest at 1890-10800 / µl and the number of *P. falciparum* parasites is 5707-15613 /µl. p-LDH/HRP2-RDT Pf / Pan in this study can detect *P. falciparum* parasites in the blood by the number of> 5000 parasites / µl of blood, *P. vivax* > 1000 parasites/ µl of blood.

Thirty two of the fifty-five negative RDT results, giving the positive results in the nested PCR examination (false negative RDT) in table 1, might be due to: a) low number of parasites in the blood. Previous research has found that pregnant women with microscopic examination is negative, but RT-PCR examination give the positive results, the number of parasites in the blood is very low at 2.9 parasites / ìl[19]; b) the type of p-LDH antigen produced by *Plasmodium* besides *P. falciparum* giving more lower sensitivity than HRP-2 antigen on the amount of blood parasites 1-50/ìl (SD Malaria Antigen Pf / Pan®)[19], so the majority of malaria infections in the number of parasites in the blood 1-50/ìl in this study was not detected by RDT; c) for the HRP-2 antigen types produced by *P. falciparum*, although the sensitivity was high 93.8%, but other factors that may affect the sensitivity of HRP-2 antigen is the possible due to the variability of antigen structure of HRP-2 antigen deletion or existence HRP-2 antigen mutation[5]. Previous research has also found a mutation of the HRP-2 antigen into HRP-3 after the sequence[7]. Antigen HRP-2 mutations can cause the HRP-2 antigen in the sample could not be detected by RDT PfHRP-2. Parasite antigens produced PfHRP3 encodes amino acids alanine and histidine-rich that similar to the antigen PfHRP2 but have differences in amino acid 4. This genetic variation can affect the detection of *P. falciparum* parasites in the amount of <1000 parasites / µL[7].

Therefore, the use of PCR for diagnosis of malaria placenta remains under discussion, especially in submicroscopic condition as it is difficult to determine what have been detected by PCR. PCR is very sensitive to detected parasites nucleic acids, but it is unclear if this is a residual from a non viable sequestered parasite, or a viable parasite, or gametocyte[29].

One positive result of microscopic examination of the *P. vivax* parasite number 10800 / µL gave a negative result on the results of RDT and nested PCR which might be due to miss interpretation of the slide.

CONCLUSIONS AND RECOMMENDATIONS

Rapid Diagnostic Test (RDT) p-LDH/HRP2 (*Pf/Pan)* has the same sensitivity to the microscopic sensitivity that is 11% when compared with nested PCR as the gold standard and less sensitive (80%) than microscopic to diagnosis malaria in pregnant women in the Maluku province as an endemic malaria areas.

Research on the validity of the RDT for pregnant women is needed before the RDT tool is used to diagnose malaria for pregnant women, especially pregnant women who living in malaria endemic areas with *oligosymptoms* and the number of parasites that tend to be low in the blood. Further epidemiological research and biotechnology research to determine the presence of HRP-2 antigen variation that can affect the reliability of the RDT tool especially for the Asia Pacific region.

ACKNOWLEDGEMENTS

The author wishes to deliver great gratitude to health centers in Ambon Municipal Health Office, Savana Jaya P. Buru Health Center, Ambon Municipal Health Office Laboratory, Maternal Inpatient Unit and Obstetrics and Gynecology Inpatient Unit of Haulussy Local Hospital, Haulussy Hospital Laboratory of Ambon.

REFERENCES

1. Schantz-Dunn J, Nour NM. Malaria and Pregnancy: A Global Health Perspective. *Med Reviews in Obstetrics and Gynecology*, 2009;82(3):186-92.
2. World Health Organization. World Malaria Report. Geneva, 2007.
3. Laihad FJ. Epidemiologi Malaria di Indonesia. *Bulentin Jendela data dan Informasi Kesehatan*. Kementrian Kesehatan RI, 2011.
4. World Health Organization. *Basic Malaria Microscopy: Part I.Learner's guide:* ed 2 . WHO, Switzerland, 2010.
5. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A Review of Malaria Diagnostic Tools: Microscopy and Rapid Diagnostic Test (RDT). *American Journal of Tropical Medicine and Hygiene,* 2007;77(6 Suppl):119-27.
6. Singer LM, Newman RD, Diarra A, Moran AC, Huber CS, Stennies G, Sirima SB, *et al*. Evaluation of A Malaria Rapid Diagnostic Test For Assessing The Burden Of Malaria During Pregnancy. *The American Journal of Tropical Medicine and Hygiene,* 2004; 70(5):481-5.
7. Baker J, McCarthy J, Gatton M, Kyle DE, Belizario V, Luchavez J, Bell D, *et al*. Genetic Diversity of *Plasmodium falciparum* Histidine-Rich Protein 2 (PfHRP2) and Its Effect on the Performance of PfHRP2-Based Rapid Diagnostic Tests. *The Journal of Infectious Diseases*, 2005;192:870-7.
8. Rogerson SJ, Mwapasa V, Meshnick SR. Malaria in Pregnancy: Linking Immunity and Pathogenesis to Prevention. *American Journal of Tropical Medicine and Hygiene,* 2007;77(6 Suppl):14-22.
9. Agbor-Enoh ST, Achur RN, Valiyaveettil M, Leke R, Taylor DW, Gowda DC. Chondroitin Sulfate Proteoglycan Expression and Binding of *Plasmodium Falciparum*-Infected Erythrocytes in the Human Placenta during Pregnancy.*Journal Infection and Immunity*, 2003; 71(5): 2455-61.
10. Menzies FM, Henriquez FL. Immunomodulation by Female Sex Hormones. *The Open Infectious Diseases Journal,* 2009; 3:61-72.

11. Mack D. Malaria. In: Shetty, N., Tang, J.W., Andrews, J (Eds.): *Infectious Disease, Pathogenesis, Prevention and Case Studies.* Wiley, London, 2009;460-74.
12. Bell DR. Lecture Notes on Tropical Medicine: Malaria: ed 4, Blackwell science, 1995:3-49.
13. World Health Organization. *Good practices for selecting and procuring rapid diagnostic tests for malaria.* WHO, Geneva, 2011.
14. SD Bioline Malaria antigen Pf/Pan® -, Korea.
15. Bintang M. Biokimia Tehnik Penelitian: Tehnik biologi molekular: Polymerase Chain Reaction, Erlangga, 2010: 246-8.
16. Sutanto I, Ismid IS, Sjarifuddin PK, Sungkar S. Parasitologi Kedokteran. Edisi 4. Dept Parasitologi FK-UI, Jakarta, 2008.
17. Moody A. Rapid Diagnostic Test for Malaria Parasites. *Clinical Microbiology Reviews*, 2002; 15(1):66-78.
18. Kashif AH, Adam GK, Mohmmed AA, Elzaki SE, Abdelhalim AM, Adam I. Reliability of rapid diagnostic test for diagnosing peripheral and placental malaria in an area of unstable malaria transmission in Eastern Sudan. *Diagnostic Pathology Journal*, 2013; 8(59):1-7.
19. Kattenberg JH, Tahita CM, Versteeg I, Tinto H, Traoré-Coulibaly M, D'Alessandro U, Schallig HDFH, *et al*. *Evaluation of antigen detecting tests, PCR and microscopy for malaria diagnosis during pregnancy* [dissertation]. University of Amsterdam, Amsterdam, 2012.
20. World Health Organization. Making Rapid Diagnosis: Making it Work. *WORLD HEALTH ORGANIZATION Regional Office for the Western Pacific*, 05(January), 2003:1-58.
21. Singh B, Bobogare A, Cox-Singh J, Snounou G, Abdullah MH, Rahman HA. A genus and species-specific nested polymerase chain reaction malaria detection assay for epidemiologic studies. *American Journal of Tropical Medicine and Hygiene*, 1999; 60(4): 687-92.
22. Kyabayinze DJ, Erana JKT, Nassali M, Tumwine LK, es Rich C, *et al*. Placental *Plasmodium falciparum* malaria infection: Operational accuracy of HRP2 rapid diagnostic tests in a malaria endemic setting. *Malaria Journal*, 2011;10(1):306-17.
23. Cottrell G, Mary JY, Barro D, Cot M. Is malarial placental infection related to peripheral infection at any time of pregnancy? *American Journal of Tropical Medicine and Hygiene,* 2005; 73(6): 1112-8.
24. Sinaga R. Penilaian sensitivitas dan spesifisitas rapid diagnostic test (RDT) dengan baku emas slide darah mikroskop untuk deteksi dini malaria dalam kehamilan. Koleksi Tesis Perpustakaan UI, Jakarta, 2006.
25. World Health Organization. Malaria Diagnosis New Perspectives: Report of a Joint WHO/USAID Informal Consultation. WHO, Geneva, 2000.
26. Agomo CO, Oyibo WA. Factors associated with risk of malaria infection among pregnant women in Lagos, Nigeria. *Infectious Diseases of Poverty*, 2013;2(19):2-8.
27. Walker-abbey A, Djokam RRT, Eno A, Leke, RFG, Titanji VPK, *et al.* Malaria in pregnant cameroonian women: the effect of age and gravidity on submicroscopic and mixed-species infections and multiple parasite genotypes. *American Journal of Tropical Medicine and Hygiene*, 2005;72(3):229-35.
28. Mockenhaupt FP, Addo GB, Gaertner C, Boye R, Frickel K, Hannibal I, Karakaya F, *et al*. Detection and clinical manifestation of placental malaria in southern Ghana. *Malaria Journal,* 2006;5:119-29

29. Kattenberg JH, Ochodo EA, Boer KR, Schallig HDFH, Mens PF, *et al*. Systematic review and meta-analysis: rapid diagnostic tests versus placental histology, microscopy and PCR for malaria in pregnant women. *Malaria Journal*, 2011;10(321):1-18.

30. Lemeshow S, Hosmer DW, Klar J, Lwanga SK. Besar Sampel Dalam Penelitian Kesehatan (terjemahan) Gadjah Mada University Press, 1997.

Tropical Medicine Journal

PAU Building
Jl. Teknika Utara, Barek, Yogyakarta 55281
0274-588483, email: tropmedjournal@gmail.com
Published by Faculty of Medicine, Universitas Gadjah Mada

Instructions to the Authors

Tropical Medicine Journal is a journal devoted to the publication of original articles in all field of basic, tropical medicine and tropical medical biotechnology.

This journal is a journal in tropical medical sciences that used as the media forndissemination of original research, innovative, ideas and new hypotheses in biomedicine, both for medical development, education and application. It also welcomes perspectives articles, biomedical history abriged articles, and reviews.

Statements and opinions expressed in the articles herein are those of author(s) responsibility and not necessary those of the Editor(s), the Faculty of Medicine, or Universitas Gadjah Mada.

Tropical Medicine Journal is published in June and December by the Faculty of Medicine, Universitas Gadjah Mada.

Submission of papers

Articles shoul be submitted in both hard copy and soft copy forms or in electronic form through e-mails as attachment to: The Editor-in-Chief, Tropical Medicine Journal, Faculty of Medicine, Universitas Gadjah Mada, Sekip Utara, Yogyakarta 55281, Phone: 0274-588483, Fax: 0274-588483 E-mail: **tropmedjournal@gmail.com**

Basic requirements for articles submitted to Tropical Medicine Journal are: a) original work; b) have not been previously published and not under consideration for publication elsewhere and if accepted will not be published elsewhere; c) should have obtained approval from the Ethics Committee; d) must obtained signed informed consent from subjects for articles involving human subjects.

Referee suggestions

Upon submission, the author should provide one cover letter. In the covering letter, authors should suggest names and addresses (including e-mail) of at least three experts in the field for evaluation of article. The choice of referees will however remain with the editorial board.

Language

Tropical Medicine Journal will publish the articles in English. Editors encourage authors to submit their articles in English. Even so, when a language barrier is encountered, editors allow authors to submit their article in Bahasa Indonesia and it will be translated in English by in-house translator.

Typescripts

Articles should be neatly typed in Times New Roman, 12 pt, double-spaced on A4 format with 3 cm on all margins. Receipt of papers will be acknowledged. Authors will be informed of the referee's comments.

Article types

Three types of articles may be submitted: a) Original research article (maximum: 25 pages, 35 references); b) Review article (maximum: 40 pages, 100 references); c) Case Report article (maximum: 10 pages, 20 references)

Proofs and Reprints

Proofs of manuscript will be sent to the author for approval prior to publication. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage. Corrections should be returned to the Editor within one week. Authors of accepted article will receive 10 free off prints of their articles and can place order for additional off prints or hard copy of the journal after the acceptance of the articles.

Copyright

Submission of an article for publication implies to the transfer of the copyright from the author(s) to the publisher upon acceptance. Accepted articles become the permanent property of Tropical Medicine Journal and may not be reproduced by any means without the written consent of the Editor-in-Chief.

Manuscript preparation

The format of the typescript should be as follows:

a. ***Title and authors***: The title should be a brief phrase describing the contents of the article. The title page should include the author's full names and affiliation that marked Arabic number. The name of the corresponding author should be indicated with postal adresse, phone, fax and e-mail information.

b. ***Abstract*****:** The author should provide two abstract, in Indonesian and English language. All articles should be provided with an sbstract of between 200-300 words in one spacing. The abstract should be written in simple language with structured abstract style. Abstract should describe of the study using below headings: Introduction, Objectives, Methods, Results and Discussion, and Conclusion. Standard nomenclature should be used and abbreviations should be avoided.

c. ***Keywords*****:** A maximum of 5 keywords must be given at the end of the abstract.

d. ***Introduction***: The Introduction should provide the problem statement clearly, the relevant literature on the subject, and the proposed approach or solution.

e. ***Materials and methods*****:** The materials and methods should be clear enough to allow experiments to be reproduced. Previously published research procedure should be cited, and important modifications of it should be mentioned briefly. If the conducted research involved the use of human subjects or animal laboratory, it should be stated that the clearance from the Research Ethics Committee was obtained. The Editor may request a copy of the clearance document or informed consent form for verification.

f. ***Results and Discussion*****:** The Results should be presented with clarity and precision and explained without referring to the literature. The original and important findings should be stated. The Results should be illustrated with figures or tables where necessary but these should be kept to the minimum. The Discussion should interpret the findings in view of the results obtained against the background of existing knowledge. The Discussion should highlight what is new in the paper. Any assumption on which conclusions are made must be stated clearly

g. ***Conclusions:*** State the Conclusions in a few sentences at the end of the paper.

h. ***Acknowledgments***: The Acknowledgments should be presented at the end of the text and before the references. Technical assistance, financial support and advice may be acknowledged.

i. ***Tables:*** The tables should be kept to a minimum and be designed to be as simple as possible. Each table should be numbered consecutively in Arabic numerals and supplied

with a heading and a legend. Tables should be self-explanatory without reference to the text.

j. ***Figure:*** The figures should be numbered consecutively with Arabic numerals. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. The figures should be constructed in such a manner that they can be understood without reading the text. Appropriate symbols should be used on graphs and explained in the legends. Graphs should not duplicate results presented in tables. Title and comments of the figures and photographs should be provied on separate page using MS Word.

k. ***References***: References should be numbered consecutively in the order in which they are first mentioned in the text (Vancouver style). Identify references by Arabic number as superscript in order of appearance. A number must be used even if the author(s) is named in the text. The original nurmber assigned to the reference is reused each time the reference is cited in the text, regarless of its provious position in the text. For example :

..... it has been reported[1] .....

..... according to Sardjito[2] .....

..... Winstein & Swartz[3] conducted .....

..... by Avon *et al*.[4] .....

Authors are responsible for the accuracy and the completeness of their references. References should be listed numerically (Vancouver style) at the end of the text and in the same order that they have been cited in the text. For citation references with six or less authors, all authors should be listed, when seven or more authors only first three authors should be listed followed by et al. Journal names are abbreviated according to Index Medicus and Index of Indonesia Learned Periodicals (PDIN 1974). References to journal articles, books, chapters in books, theses, etc. should be listed as given in Sample References.

Sample References

Scientific Journal

1. *Standard journal article*

   You CH, Lee KY, Chey RY, Menguy R. Electro-gastro-graphic study of patients with unexplained nausea, bloating and vomiting. Gastroenterology 1980; 79(2):311-14.

   Goate AM, Haynes AR, Owen MJ, Farral M, James LA, Lai LY, et al. Predisposing locus for Alzheimer's disease on chromosome 21. Lancet 1989;1:352-55.

2. *Organization as author*

   The Royal Marsden Hospital Bone-marrow Transplantation. Team. Failure of syngeneic bone-marrow graft without preconditioning in post-hepatitis marrow aplasia. Lancet 1977;2:742-44.

3. *No author given*

   Coffee drinking and cancer of the pancreas [editorial]. BMJ 1981;283-628.

4. *Article not in English*

   Massone L, Borghi S, Pestarino A, Piccini R, Gambini C. Localisations palmaires purpuriques de la dermatite herpetiforme. Ann Dermatol Venereol 1987;114:1545-47.

5. *Volume with supplement*

   Magni F, Rossoni G, Berti F, BN-52021 protects guinea-pig from heart anaphylaxis. Pharmacol Res Commun 1988;20 Suppl 5:75-78.

6. *Issue with supplement*

   Gardos G, Cole JO, Haskell D, Marby D, Paine SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

7. *Volume with part*

   Hanly C. Metaphysics and innateness: a psychoanalytic perspective.Int J Psychoanal 1988;69(Pt 3):389-99.

8. *Issue with part*

   Edwards L, Meyskens F, Levine N. Effect of oral isotretinoin on dysplastic nevi. J Am Acad Dermatol 1989;20(2 Pt 1):257-60.

9. *Issue with no volume*
   Baumeister AA. Origins and control of stereotyped movements. Monogr Am Assoc Ment Defic 1978; (3):353-84.
10. *No issue or volume*
    Danoek K. Skiing in and through the history of medicine. Nord Midicinhist Arsb 1982;86-100.
11. *Pagination in roman numerals*
    Ronne Y. Ansvarfall. Bloodtransfusion till fel patients. Vard-facket 1989;13:XXVI-XXVII.
12. *Type of article indicated as needed*
    Spargo PM, Manners JM, DDAVP and open heart surgery [letter]. Anaesthesia 1989;44: 363-64.
    Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondii [abstract]. Clin Res 1987; 35:475A.
13. *Article containing retraction*
    Shishido A. Retraction notice: Effect of platinum compounds on murine lymphocyte mitogenesis [Retraction of Alsabti EA, Ghalib ON, Salem MH. In: Jpn J Med Sci Biol 1979; 32:53-65). Jpn J Med Sci Biol 1980;33:235-37.
14. *Article retracted*
    Alsabti EA, Ghalib ON, Salem Mh. Effect of platinum compounds on murine lymphocyte mitogenesis [Retracted by Shishido A. In: Jpn J Med Sci Biol 1980;33:235-7]. Jpn J Med Sci Biol 1979;32:53-65.
15. *Article containing comment*
    Piccoli A, Bossatti A. Early steroid therapy in IgA neuropathy: still open question [comment]. Nephron 1989;51:289-91.
16. *Article in comment*
    Kobayashi Y, Fujii K, Hiki Y, Tateno S, Kurokawa A, Kamiyama M. Steroid therapy in IgA nephropathy: a retrospective study in heavy proteinuric cases [see comments]. Nephron 1988;48:12-7. Comment in: Nephron 1989;51:289-91.
17. *Article with published erratum*
    Schofield A. The CAGE questionnaire and psychological health [published erratum appears in Br J Addict 1989;84:701]. Br J Addict 1988;83:761-64.

Books and Other Monographs

18. *Personal author(s)*
    Colson JH, Armour WJ. Sports injuries and their treatment. 2nd rev. ed. London: S. Paul, 1986.
19. *Editor(s) as author*
    Diener HC, Wilkinson M, editors. Drug-induced headache. New York: Springer-Verlag, 1988.
20. *Organization(s) as author*
    Virginia Law Foundation. The medical and legal implications of AIDS. Charlottesville: The Foundation, 1987.
21. *Chapter in a book*
    Winstein L, Swartz MN. Pathologic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, editors. Pathologic Physiology, mechanisms of disease. Philadelphia: Saunders, 1974:457-72.
22. *Conference proceedings*
    Vivian VL, editor. Child abuse and neglect: a medical community response. Proceedings of the First AMA National Conference or Child Abuse and Neglect; 1984 Ma 30-31; Chicago. Chicago: American Medical Association, 1985.
23. *Conference paper*
    Harley NH. Comparing radon daughter dosimetric and risk models. In:Gammage RB, Kaye SV, editors. Indoor air and human health. Proceedings of the Seventh Life Sciences Symposium; 1984 Oct 29-31; Knoxville (TN). Chelsea (MI):Lewis, 1985:69-78
24. *Scientific or technical report*
    Akutsu T. Total heart replacement device. Bethesda (MD): National Institutes of Health. National Heart and Lung Institute; 1974 Apr. Report No.:NIH-NIHI-69-2185-4.
    Disertasi Youssef NM. School adjustment of children with congenital heart disease [dissertation]. Pittsburg (PA): Univ. of Pittsburg, 1988.

25. *Dissertation*
    Kay JG. Intracellular cytokine trafficking and phagocytosis in macrophages [Dissertation]. St Lucia, Qld: University of Queensland; 2007.
26. *Patent*
    Harred JF, Knight AR, McIntyre JS, inventors. Dow Chemical Company, assignee. Epoxidation process. US patent 3,654,317, 1972 Apr 4.

Other Published Material

27. *Newspaper article*
    Resberger B, Specter B. CFCs may be destroyed by natural process. The Washington Post 1989 Aug 7;Sect. A:2(col. 5).
28. *Audiovisual material*
    AIDS epidemic: the physician's role [videorecording]. Cleveland (OH): Academy of Medicine of Cleveland, 1987.
29. *Computer program*
    Renal system [computer program]. MS-DOS version. Edwardsville (KS): Medi-Sim, 1988.
30. *Legal material*
    Toxic Substances Control Act: Hearing on S. 776 Before the Subcomm. on the Environment of the Senate Comm. on Commerce, 94th Cong., 1st Sess. 343(1975).
31. *Map*
    Scotland [topographic map]. Washington: National Geographic Society (US), 1981.
32. Dictionary or Encyclopaedia
    Ectasia. Dorland's illustrated medical dictionary. 27th ed. Philadelphia: Saunders, 1988: 527.
33. *Classic material*
    The Winter's Tale: act 5, scene I, lines 13-16. The complete works of William Shakespeare. London: Rex, 1973.
34. *In press*
    Lillywhite HB, Donald JA. Pulmonary blood flow regulation in an aquatic snake. Science. In press.

Electronic Material

35. *Journal articel in the internet*
    Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis [serial online] 1995 Jan-Mar [cited 1996 Jun 5];1(1):[24 screens]. Available from: URL: http://www.cdc.gov/ncidod/EID/eid.htm
36. *Monograph in electronic format*
    CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reeves JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0 San Diego: CMEA; 1995.
37. Computer program
    Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational System; 1993.

We thank to the reviewers of this edition:

dr. Abu Tholib, M.Sc., Ph.D., Sp.MK
dr. Ahmad Hamim Sadewa,Ph.D
dr. Arta Farmawati, Ph.D
dr. Elizabeth Henny Herningtyas, M.Si, Ph.D
Dr. Dra. Erna Kristin, Apt., M.Si.
dr. Hanggoro Tri Rinonce, Ph.D
dr. M. Lutfan Lazuardi, M.Kes., Ph.D
dr. Titik Nuryastuti, Ph.D, Sp.MK
dr. Tri Wibawa, Ph.D
Dra. Raden Ajeng Yayi Suryo Prabandari, M.Si., Ph.D