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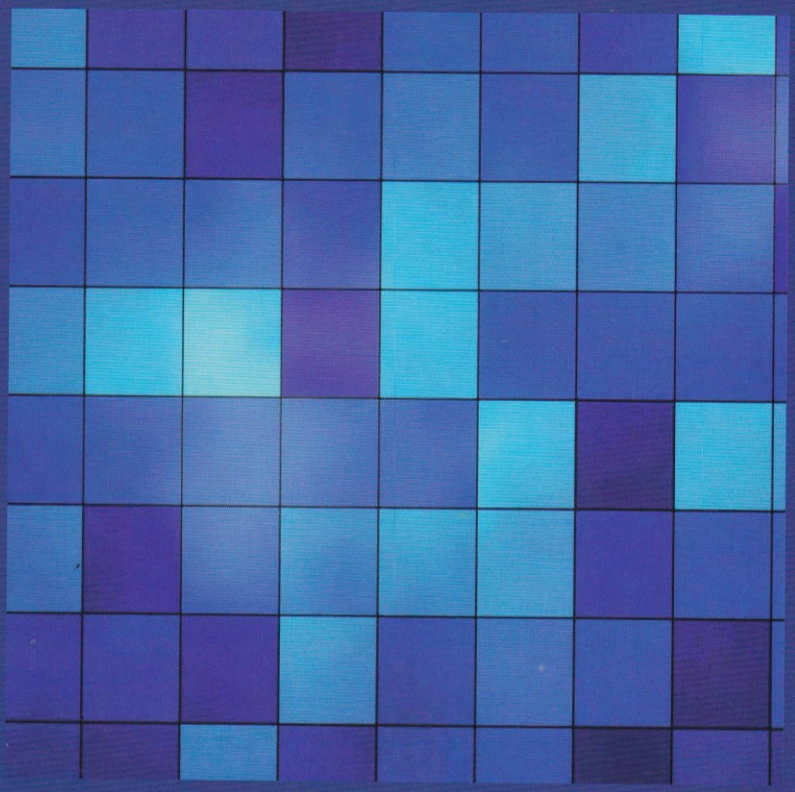

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Article:

Sensitivity and specificity EXAMINATION Rapid Diagnostic Test (RDT) and microscopic examination on Plasmodium falciparum

Mariana Hanafiah

Sriwijaya University-Indonesia

Signature

THEME
MACROJOURNALS

Dr. Damir Tokic
Conference Chair

Presented on December 19-20th, 2014, in Paris, France
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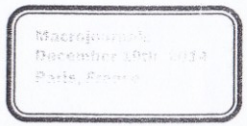

The MacroTrend
Conferences, Paris 2014

The Journal of MacroTrends in Health and Medicine

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October 2, 2014

Mrs. Mariana
Sriwijaya University-Indonesia
Abstract Title: : Sensitivity and specificity EXAMINATION Rapid Diagnostic Test (RDT) and microscopic examination on Plasmodium falciparum

Subject: Letter of acceptance (invitation)

Dear Mrs. Mariana,

Your article/abstract has been peer-reviewed and accepted for an oral presentation (or poster if requested) at **the MacroTrend Conference on Health and Medicine: Paris 2014**, which will be held on December 19-20, 2014 in Paris, France.

The conference venue is: ESPACE VOCATION PARIS HAUSSMAN SAINT-LAZARE 92, rue Saint-Lazare 75009 Paris. Publishing opportunity for full papers: *The Journal of MacroTrends in Health and Medicine*. All abstracts will be published in the conference proceedings. The conference registration fees are $450 ($370 students and $250 for each attending co-author). Please visit our payment site for more info: http://www.macrojournals.com/payments. Also, please visit the conference webpage for more info about the venue area for booking a hotel, and important dates/deadlines: http://www.macrojournals.com/paris/health_and_medicine

We welcome you to the conference and looking forward to your intellectual contribution.

Best regards,

Dr. Damir Tokic

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The MacroTrend Conferences, Paris 2014

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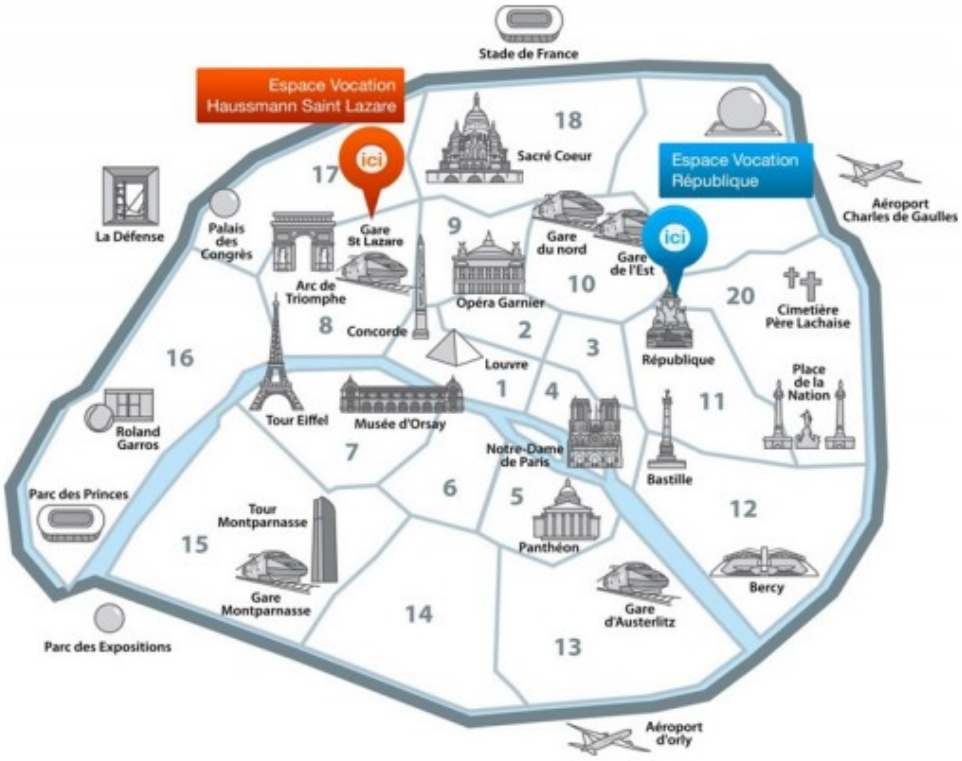

Session 1: Health and Medicine Dec 19th 2014, Room TBA

9:00am **CPAP improves daytime sleepiness, functional outcomes and daily body energy expenditure in obstructive sleep apnoea**
A Mehdi 1, G. Baker2, T. Van der Touw 1
1School of Science and Technology and 2School of Rural Medicine University of New England. Armidale, Australia.

9:15am **Efficacy and safety of a novel CdSe/L-Cys quantum dots for investigation of pathology of women reproductive system: in vitro analysis**
Anna.O. Durnova, Yu.S. Krylova, S. F. Musikhin, L.B. Matyushkin, V. A. Moshnikov, O.A.
Aleksandrova, D.S. Masing, V.O. Polyakova, I. M. Kvetnoy.
Ott Research Institute of Obstetrics and Gynecology,
Saint Petersburg Electrotechnical University,
Saint-Petersburg Polytechnical University

9:30am **Steroidogenic enzymes, their related transcription factors and nuclear receptors in human sebaceous glands under normal and pathological conditions**
Abdullah Azmahani a,b, Yasuhiro Nakamura a,*, Saulo J.A. Felizola a, Yohei Ozawa a,c, Kazue Ise a, Takayoshi Inoue d, Keely M. McNamara a, Masao Doi e, Hitoshi Okamura e, Christos C. Zouboulis f, Setsuya Aiba g and Hironobu Sasano a
Department of Pathology, Tohoku University Graduate School of Medicine, Sendai, Japan; Faculty of Medicine and Health Sciences, University Sultan Zainal Abidin, Kuala Terengganu, Terengganu, Malaysia; cDivision of Advanced Surgical Science and Technology, Tohoku University Graduate School of Medicine, Sendai, Japan; dBiological Science Laboratories, Kao Corporation, Haga, Tochigi, Japan; eDepartment of Systems Biology, School of Pharmaceutical Sciences, Kyoto University, Kyoto 606, Japan; Departments of Dermatology, Venereology, Allergology and Immunology, Dessau Medical Center, Dessau, Germany; Department of Dermatology, Tohoku University Graduate School of Medicine, Sendai, Japan.

9:45am **The role of the Unknown Bacterial Agent in the Etiology and Pathogenesis of Primary Liver Cancer**
Alexandre TAVARTKILADZE., Givi TAVARTKILADZE
Georgian Cancer and Internal Medicine Research Center

10:00am **FIXED DOSE DRUG COMBINATIONS IN INDIA- IS THERE ENOUGH SCIENTIFIC EVIDENCE**
Dahiya Akhil
Maulana Azad Medical College & Associated Lok Nayak, GB Pant Hospitals, New Delhi, India

10:15am **Is there enough evidence for intermittent iron supplementation?**
Sneha Kaushik
Vardhman Mahavir Medical College & Safdarjang Hospital, New Delhi, India

10:30am

The effect of participatory learning program prevention of soil-transmitted helminth (STH) infections among ethnic minority group in primary students Nan Province, Thailand
Katekaew Seangpraw, Surasak Taneepanichskul, Ratana Somrongthrong
College of Public Health Sciences, Chulalongkorn University, Thailand

10:45am **Medical lab Professionals' Perception's about Continuous Medical Education in Security Forces Hospital, Riyadh, KSA**
Samar Sami Qasim
Bsc medical laboratory from King Saud University - Riyadh
Msc Health &Hospital Administration from King Saud University - Riyadh
Serology - Immunology & Molecular Biology Section Head
Laboratory & Blood Bank Department
Security Forces Hospital KSA -Riyadh

11:00am **Engagement of Medical Aesthetic Clinicians in a Health Educational Campaign to Increase Public Knowledge and Awareness on Safety & Efficacy of Over the Counter Products Labeled by Stem Cell**
Farahnaz Amini, Goh Pei Teng, and Ng ChiatYin
School of Anti-Aging, Aesthetics and Regenerative Medicine, Faculty of Medicine and Health
Science, UCSI University, Kuala Lumpur, Malaysia

11:15am **Anti-Hyperglycemic Effect of Duku (Lansium domesticum Corr) Seed Extract in Alloxan Induced Diabetic Rats**
Iche Andriyani Liberty
Sriwijaya University-Indonesia

11:30am **Sensitivity and specificity EXAMINATION Rapid Diagnostic Test (RDT) and microscopic examination on Plasmodium falciparum**
Mariana Hanafiah
Sriwijaya University-Indonesia

11:45am **It's time to blink, Eye blink sensor: A boon to occupational dry eye syndrome**
Vikas Verma1 ,Charu Kohli2 , Shantanu Sharma3,
1BioMedical research Engineer,3,2Department of Community Medicine, Maulana Azad Medical College, New Delhi, India,1Consultant Engineer,

12:00 noon **The effect of Swedish massage on blood pressure in patients**
Nariman Sadeghi Kaji
Iran

Discussant(s):
Chi-Fu Jeffrey Yang,
Duke University Medical Center, USA

Research Article

Sensitivity and specificity EXAMINATION Rapid Diagnostic Test (RDT) and microscopic examination on *Plasmodium falciparum*

Mariana, Chairil Anwar, Theodorus

Faculty of Medicine, University of Sriwijaya, Palembang

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Abstract

Malaria is still becoming healthy problem in tropical country. Malaria cases in the province of Bangka Belitung still above 1/1.000. The highes influences is on west, south and east Bangka. The highest rate of malaria found in the West Bangka, about 86.87 per 1000 population. However, the availability of expert microscopic examination is limited. Therefore it is important to find alternative examination in this province because of the highest rate of malaria infection.The purpose of research is to investigate the sensitivity, specificity, positive predictive value, and negative predictive value by using the method of examination of *Plasmodium falciparum Rapid Diagnostic Test* (RDT) and microscopic at General Hospital West Bangka distric. The testing of these two sensitivity test has been done in the Hospital of the West Bangka Sejiran Setason from April 1st, 2013 to April, 20th 2013. In 134 samples by using purposive sampling based on consideration that fulfil the inclusion criteria of the researcher. The sample was taken by Passive Case Detection (PCD). The results from 134 respondents were male respondents was about 68 (50.75%) and, more in adulthood at 85 (63.43%), with un educated respondent is aout 47 (35.07%), and the level of employment of farmers about 71 respondents (52.99%). By using microscopic the result was 8 positive samples and as many as 126 samples were negative. By using RDT showed zero positive in the sample and negative results was 134 samples, therefore the sensitivity in this research was 0.0002%, specificity 100%, positive predictive value of 0.5%, and negative predictive value 94.02 %. The conclusions obtained in this research was that there still the limitation skilled laboran, RDT was still becoming an alternative, but it was need to have further research with bigger number of sample by using other type RDT. Therefore it can be decided which RDT was perfectly implemented on such area

Keywords: Plasmodium falciparum, PfHRP II, RDT.

1. Introduction

Malaria is an infectious disease caused by the malaria parasite, is transmitted to humans through the bite of the Anopheles mosquito. This disease can affect all races, ages and genders. Malaria is endemic in some areas. Various species of the genus Plasmodium of the class Sporozoa are parasitic in humans (Irianto, 2009). Malaria is still a health problem in tropical countries in the world. It is estimated that approximately 40 percent of the world's population still lives in areas that are at high risk for malaria infection with 500 million clinical cases and 1 million deaths per year due to malaria (WHO, 2007).

Annual Malaria Incidence (AMI) / 1000 population in Bangka Belitung in 2007 was 42.14. At the present time cases of malaria in Bangka Belitung province is still above 1/1000. It is expected that by 2014 the number could be below 1/1000 cases. The area is still a high number of cases is West Bangka, Bangka Belitung South and East. Malaria morbidity are highest in the West Bangka Regency which amounted to 86.87 per 1000 population (Health Profile Bangka Belitung Islands, 2007). In addition to AMI, the Annual Parasite Insidence (API) in Bangka Belitung Islands also still high at 40.58 per 1000 population (Health Departemen, 2008). There are two ways to determine whether the patients were positive for malaria by microscopy and Rapid Diagnostic Test (RDT). Until now the gold standard for the diagnosis of malaria is by blood tests with a thick or thin Giemsa staining were examined by light microscopy. The advantage of coloring is that it has a high sensitivity. It shows Giemsa staining was able to detect malaria parasites, although at a low density. In addition, Giemsa staining can also calculate the burden of malaria parasites and distinguish species and the stage (Basundari, 2004).

Microscopic examination is highly dependent on the expertise of the laboratory institutions (health analyst) is to identify, therefore misdiagnosis may occur and will lead to inappropriate treatment that can be fatal. An expert is able to detect microscopic parasites although the numbers are less than 50 parasites /mL of blood. But those who have high ability as this is still very little. Meanwhile, to get a qualified person, takes time because it requires intensive training and long experience in the microscopic examination (Joyce, 2007).

RDT is an alternative to the established diagnosis based on clinical manifestations, especially in places that have no means of microscopic quality. Although there are various types of RDT, but the same principle, namely to detect specific antigens (proteins) produced by the malaria parasite and are in the infected person's blood circulation. Several RDTs can detect only one species of Plasmodium while others can detect multiple species of Plasmodium. RDT is the advantage of this examination does not require high expertise for implementation (Aphiah, 2009).

With a high level of endemicity, West Bangka district requires accurate diagnostic techniques. However, the availability of expert microscopic examination is very limited. This calls for alternative examination given the magnitude of the health problems associated with malaria while a wide range of available screening methods still contains many weaknesses in delivering the diagnosis, it is necessary to investigate how the sensitivity and specificity of the diagnosis of Plasmodium falciparum by RDT method. The results of this study are expected to be the basis for selecting the method of diagnostic tests in malaria endemic areas.

Research Objectives

1 General Purpose

To determine the sensitivity and specificity of Plasmodium falciparum using the method of Rapid Diagnostic Test (RDT) and microscopic methods in the General Hospital of the West Bangka Regency.

2 Special Purpose

To determine the positive predictive value (NPP) using the method of diagnosis of Plasmodium falciparum Rapid Diagnostic Test (RDT) and microscopic methods in the General Hospital of the West Bangka Regency.

3 To determine the predictive value of a negative (NPN) using the method of diagnosis of Plasmodium falciparum Rapid Diagnostic Test (RDT) and microscopic methods in the General Hospital of the West Bangka Regency.

4 Research Methods

A. Types of Research
   This study is a diagnostic test that compare RDT method and microscopic examination as a gold standard for Plasmodium falciparum.

B. Place and Time Research
   1 place In this study, blood samples were taken from the blood of patients who received laboratory through a common poly and ER (Emergency Room) Sejiran Setason Hospital West Bangka.

   2 Time The study began with a search list of libraries, initial survey, preparing research proposals, designing questionnaires, conducting research and preparation of the final report. This study was carried out for 8 months since the search literature, seminars and comprehensive exam results, was from November 2012 until June 2013.

C. Population and Sample
   1 Population Research The population is suspected malaria all who came to the laboratory and has been examined by a doctor at the General poly and IGD.
   2 Study Sample The samples were suspected malaria who met the inclusion criteria.

D. Sampling Method Sampling research conducted purposive sampling, which is based on the consideration that met the inclusion criteria of the researcher (Lameshow et al, 1990).

E. Procedures
   1 Microscopic Methods Specimens: peripheral blood is used, taken from the tip of the ring finger / middle left hand. For infants 6-12 months, were taken from the tip of the big toe and aged less than 6 months should be taken from the heel of the foot.

When using venous blood, blood should be used is the blood that has not been mixed with an anticoagulant.

a. Tools and materials Glass cleaner preparations a. lancet
b. Cotton alcohol and a cotton dry
c. Microscope and oil immersion
d. Giemsa stock solution
e. Ways capillary blood sampling and manufacture of blood smear.

1) Hold the patient's left hand with palms facing up.
2) Select the middle finger or sweet (in infants aged 6-12 months of blood is drawn from the tip of my toes and infants <6 months of blood taken from the heel)
3) Clean the finger with cotton alcohol to remove dirt and oil that sticks to the fingers.
4) Once dry, finger pressed to accumulate a lot of blood on the fingertips.
5) Stick this part fingertip (somewhat to the side, near the nail) quickly using the lancet.
6) drops of blood which came out first cleaned with dry cotton to remove the blood clot and the residual alcohol.
7) Press the fingertips until blood came out, grab a clean glass objects (objects hold the glass section edges). The position of the glass object is under the finger.
8) Put 1 drop of blood in the center of the object glass for blood clots (SD) thin. Furthermore, 2-3 drops of blood were greater for elementary thick.
9) Clean the rest of the blood at the tip of the finger with the cotton.
10) Put a glass object which contains drops of blood on a table or flat surface.
11) To make blood preparations (SD) thin, take the new glass object but not the cover glass. Glue ends in a small drop of blood until the blood spreads throughout the object glass.
12) With a 45o angle of the glass object slide quickly in the opposite direction with thick drops of blood, so we get a clear preparations (such as the shape of the tongue).
13) To SD thick, the second end attached to a glass object to three drops of blood thick. Blood is made homogeneous by rotating the tip of glass objects clockwise, thus forming a sphere with a diameter of 1 cm.
14) primary drying process must be done slowly in a flat. Not recommended to use light (including light microscopy), hair dryer. This can be the primary cause cracks that affect the outcome of the examination. The fan can be used for drying SD.
15) During the drying process, SD should be protected from insects (ants, flies, cockroaches, and others), dust, heat, high humidity and vibration. 16) After drying, the blood had to be colored. In the circumstances do not allow no later than within 24 hours of SD should already be colored (Health Departement, 2009).

f. Analysis of Data

Data already collected grouped, edited, coded and then performed data processing. The research data presented in tables and narrative. Then the data were analyzed by the method of analysis Medcalc.

5. Results

a. characteristics of respondents

1) Gender

Table 1.Distribution of respondents by gender (n=134)

Gender	Number (people)	Percentage (%) (%)
Male	68	50,75
Female	66	49,25
Amount	134	100,00

2) Age

Tabel 2. Distribution by age

Age (Year)	Number (People)	Percentage (%)
0-14	32	23,88
15-50	85	63,43
>51	17	12,69
Amount	134	100,00

3) Education

Table 3.Distribution by Education

Education	Number (People)	Percentage (%)
-(not school)	32	35,07
Elementary	85	17,17
Junior High School	17	23,13
Senior High School		21,64
University		2,99
Amount	134	100,00

4) Occupation

Table 4. Distributin by Occupation

Pekerjaan	Jumlah (Orang)	Persentase (%)
Civil Servant/Polri/TNI	1	0,74
Private/Entrepreneurial	12	8,96
Workers/Farmers	71	52,99
Miners	18	13,43
Not Working	32	23,88
Amount	134	100,00

5) Microscopic (Giemsa)

Table 5. Distribution by Microscopic Result Test

Microscopic/ Giemsa	Number (People)	Percentage (%)
Positive	8	5,97
Negative	126	94,03
Amount	134	100,00

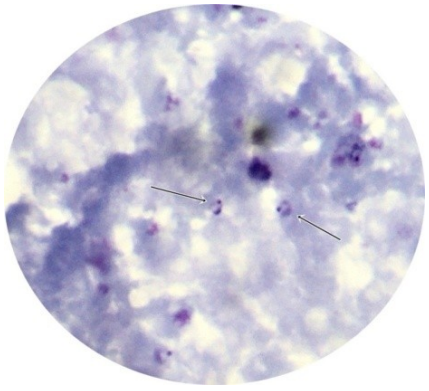

Image. Positive Result of *P. falciparum* on Microscopic. Tropozoit ringform Stadium

6) RDT result

Table 6. Distribution by RDT Result Test

RDT	Number (People)	Percentage (%)
Positive	0	0
Negative	134	100.00
Amount	134	100,00

7) Result Microscopic Diagnostic Tes and RDT

Table 7. Result Microscopic Diagnostic Tes and RDT

		Microscopic Result		
		Positif	Negatif	Amount
RDT Result	Positive	0	0	0
	Negative	8	126	134
	Amount	8	126	134

From Tablel 7 above, the result are:

Sensitivitas = 0,0002%

Spesifisitas = 100%

NPP = 0,5%

NPN = 94,02%

2.Discussion

In Gender appears that male respondents was more than female respondents. These results are in accordance with the study conducted by Ritawati and Yahya (2012) in which the male respondents (51%) more often than female respondents (49%) it is probably because men do more activities outside the home to look for living. Thus the number of Anopheles mosquitoes that bite them higher than women.

Characteristics of respondents according to age of the study with similar results was performed by Desrinawati (2003) in which respondents aged adults (15-50 years) is the most respondents (50%) this can be caused by adult respondents with various activities outside the home, especially in places mosquito breeding in dark or night time, would be allowed to contact with mosquitoes so that the malaria transmission can be.

The results based on the education level of school education level was not / is not finished, this study is also consistent with the results of the study Romi (2006) who get most respondents at the level of school education is not 93.03% of the data that has been obtained is seen that most respondents most respondents to the level of education is not school, this high number may be due to the respondents' knowledge about malaria is low and therefore contributes to the occurrence of malaria transmission such as sleep habits do not use mosquito nets, do not wear mosquito repellent when outside the home and often outside home at night without closing the body can be a risk factor for malaria transmission.

In research conducted by Renny (2011), showed similar results where respondents were highest in the group of workers / farmers were 21 (36.3%). This can be caused by many land which is vacant land that many residents who grow crops in the area, residents in the district of the West Bangka many farmers pepper, rubber and oil palm farmers, and many of those who live in areas that are many puddles in the back of people's houses as household waste disposal sites, mixed gardens, bushes, marshes and ponds are not maintained and provides a possible breeding places of malaria.

A positive result is found microscopic 8 respondents (5.97%) were positive, but results of studies Basundari (2004) gave results of 9.24% of this difference may be due to the low number of parasites in the blood smear preparations that are undiagnosed. The different results obtained in the study conducted by Basundari et al (2008) who received 4 positive results from 106 samples of Plasmodium falciparum, researchers are getting 0 positive results of 134 samples, the low parasite density and mutation of a gene called HRP-2 mostly as a result cause false-negative malaria RDT for P. falciparum. Prozone effect might be an alternative explanation for the false-negative results at high parasite densities. The prozone-effect (also known as the phenomenon of high-dose hook) is defined as negative or false low results in immunological reactions, because an excess of either antigen or antibody. In this case, a high concentration of antigen which will block all the available bonds of both detection and capture antibodies, thus inhibiting the binding of antigen and antibody detection antibody complex conjugate to the catcher .. Of the 8 cases with a positive malaria blood slide microscopy examination, not none were detected with RDTs used in this study, so the sensitivity in this study was 0.0002%. But the WHO recommendations state that a good RDT should have high sensitivity and specificity (> 95%), manufacturers RDT kits used in this study also suggested that the ability of the sensitivity of the RDT kits were> 99.0% so it should of 8 samples with a positive blood slide microscopy rnemberikan least nothing positive with RDT results in this research. The reduced sensitivity of RDTs may be influenced types of parasites and levels of parasitemia, but based on the calculation of the number of parasites to 8 respondents result is> 500 / uL should have a sensitivity of 90%, a negative RDT result may be due to the influence of parasite strains as well as the target antigen polymorphism PFHRP2 Theoretically, presence and absence, as well as the number of repetitions PfHRP2 may affect the binding affinity of the antibody used in the RDT with parasite antigens. PfHRP2, the most common targets of malaria RDTs, highly polymorphic throughout malaria-endemic areas.

While this may affect the sensitivity of RDTs on parasite density is very low. Wide diversity in order pfhrp2 also provide evidence that there is no strong evolutionary selection for any particular type of sequence (Baker et al, 2010). Cross reaction between PfHRP3 also thought to be a cause of false-negative results in the RDT. antibodies present in the RDT-based cross-react with antigens PfHRP2 other parasites. The most likely candidate for the antigen is PfHRP3. PfHRP3 encodes 4 amino acids repeated, alanine and histidine rich protein that is identical to the one in PfHRP2 (types 1, 2, 4, and 7) and 4 different. It is entirely possible that either some or all of the epitopes detected by the antibody used in the RDT also exist in PfHRP3, the results of the analysis that the repetition of type 2 and type 7 may be involved in cross-reactivity.

Various levels of cross reaction between PfHRP2 and PfHRP3 also been reported. Antisera against peptides PfHRP2 and PfHRP3 particularly recognize the appropriate proteins but also recognize the other weak. It is also possible that other parasite histidine rich protein can be recognized by the monoclonal antibody PfHRP2 (Baker et al, 2005). The second factor that can modulate the effects of genetic diversity PfHRP2 is that the RDT using two antibodies: one acts as a signal antibody and 1 acts as a capture antibody / catcher. Both antibodies recognize epitopes in PfHRP2. If the two antibodies recognize different epitopes, the reduced number of epitopes can be compensated by a large number of second epitope. The combined effect of cross-reactivity between PfHRP2 and PfHRP3 and the inclusion of two different antibodies in the RDT can help detect parasite density> 1,000 parasites / FL. Genetic diversity seems to primarily affect detection PfHRP2 at parasitemias <1000 parasites / FL. The majority of commercially available RDTs based on the detection PfHRP2 and identify only P. falciparum. No published information is available on the target-specific epitope of antibody used in one of these RDTs or the 6 mAbs directed against PfHRP2 published [38, 41-43]. Because of the limited supply of mAbs, it is likely that most of the RDT-based PfHRP2 using similar or identical antibodies. Such data will allow us to use sequence information to better predict PfHRP2 potential effects of genetic diversity on performance of RDT and RDT design is more sensitive to the use of a more sensitive mAbs, the genetic background of the parasite must be taken into account when the device is tested in a field setting or sensitivity when compared between different settings or assessed against local endemic parasites (Baker et al, 2005).

RDT use is further limited by the stability of the interference caused by temperature fluctuations during transport and storage facilities that are not controlled in the field based. RDT kits require storage temperatures between 2-30 ° C, but in this study the problem could be ignored as long as the trip was taken in the RDT kit box containing ice cubes for cooling and kept in a room with a temperature of 16 ° C.

The results of the diagnostic test with Plasmodium falciparum RDT method compared with the microscopic method obtained 0.0002% sensitivity, 100% specificity, positive predictive value of 0.5% and a negative predictive value of 94.02%. RDT specificity was 100% so it is not possible respondents not diagnosed malaria malaria. Negative predictive value of 94.02% so that a negative result in respondents with RDT

examination showed respondents most likely really do not suffer from malaria. With the results of the sensitivity and positive predictive values were low, and the specificity and high negative predictive value of this method ideally only for screening tests. The results of this study is similar to a 2006 study Romi getting the sensitivity of 0%, specificity 100% 0% positive predictive value, negative predictive value of 91.1%. Based on the results of this diagnostic examination RDTs used in this study are used for a screening (penafisan), is not accurate for the detection of Plasmodium falciparum parasites and microscopic examination of blood slides is still the gold standard. PfHRP2 genetic diversity and its relationship with MABS ability to bind to a serious challenge for future testing and development that target HRP2 RDTs for the diagnosis of Plasmodium infection. falciparum.

Acknowledgement

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