The comparative accuracy of rapid diagnostic test with microscopy to diagnose malaria in subdistrict Lima Puluh Batubara regency North Sumatera province

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Abstract. Indonesia is the country where malaria is still the most common population problem. The high rate of mortality and morbidity occurred due to delays in diagnosis which is strongly influenced by the availability of diagnostic tools and personnel with required laboratory skill. This diagnostic study aims to compare the accuracy of a Rapid Diagnostic Test (RDT) without skill requirement, to a gold standard microscopic method for malaria diagnosis. The study was conducted in Subdistrict Lima Puluh North Sumatera Province from December 2015 to January 2016. The subject was taken cross-sectionally from a population with characteristics typically found in malaria patients in Subdistrict Lima Puluh. The result showed a sensitivity of 100% and a specificity of 72.4% with a positive predictive value of 89.9% and a negative predictive value of 100%; the negative likelihood ratio is 0 and the positive likelihood ratio of 27.6 for Parascreen. This research indicates that Parascreen had a high sensitivity and specificity and may be considered as an alternative for the diagnosis of malaria in Subdistrict Lima Puluh North Sumatera Province especially in areas where no skilled microscopist is available.

1. Introduction
Malaria is a global health problem with 207 million cases and more than 627,000 deaths annually, especially for children under five years old in Sub-Saharan Africa. The World Health Organization (WHO) also records 300 - 500 million people infected with malaria each year. Malaria is also a health problem in more than 90 countries, accounting for 40% of the world's population. As many as 90% of the malaria incidence occurs in Sub-Saharan Africa. Asia ranks second after Africa. WHO estimates about 34.8 million cases and 45,600 deaths from malaria in Asia and reported more than 85% of malaria and deaths occur in India, Indonesia, Myanmar, and Pakistan.[1-3] The types of Plasmodium found in Indonesia are \textit{Plasmodium falciparum} (\textit{P. falciparum}) and \textit{Plasmodium vivax} (\textit{P. vivax}). \textit{P. falciparum} causes the deaths of over 600,000 cases per year.[2] The high rate of morbidity and mortality from malaria occur due to delays in diagnosis and antimalarial drug resistance.[4] Delay in diagnosis is strongly influenced by the availability of diagnostic tools in a particular area. [5,6,7]
Shortage of medical laboratory workers skilled in using microscopes to diagnose malaria properly is one of the causes of treatment delay and malaria misdiagnosis. One alternative method of examination that is relatively easy to use is Rapid Diagnostic Test (RDT).[3,8,9,10]

2. Materials and Methods

2.1. Location, time and population
This research was conducted from December 2015 to January 2016 in Lima Puluh Subdistrict, Batubara Regency, North Sumatera Province, Indonesia. Lima Puluh District is the largest district in Batubara Regency which is located on the east coast. Specifically, it is located in the lowlands and is generally close to the beach. The frequent occurrence of sea tides causes puddles to form and garbage is usually scattered all over the ditch. In addition, abandoned shrimp aquaculture becomes vector-breeding sites. This study used a diagnostic test with cross-sectional approach. The research targeted a population that has characteristics typically found in malaria patients in Lima Puluh District. The populations that are within reach are subjects located in three villages in Lima Puluh District. Screening all people with criteria fever ≥ 37.5 °C with or without documented fever within 48 hours.

2.2. Data collection
Rapid Diagnostic Test (RDT) and blood smear examination (microscopic) were performed all at once, and both tests were respectively labeled and placed at different spots for examination.

Interviews were performed and questionnaires were completed by patient or patient’s guardian; anamnesis and physical examination in which their body temperature. These examinations were conducted at the same time and are respectively labeled and placed in different spots in which microscopic result are not influenced by the results of RDT announcement. Rapid diagnostic tests were examined using Parascreen™ devices produced by Zephyr Biomedicals, India. The rapid diagnostic tests malaria positive for malaria falciparum and or Pan (other malaria species such as P. vivax, Plasmodium ovale, or Plasmodium malariae). The result can be interpreted within 15 minutes. RDT was performed by trained researchers and paramedics. Thick and thin peripheral blood films were obtained, stained with Giemsa and examined microscopically under oil immersion. The collected blood were confirmed by trained researchers and parasitologist at the laboratory of Faculty of Medicine University of Sumatera Utara.

2.3. Ethics
Ethical clearance was approved by Ethical Committee of Medical Faculty, University of Sumatera Utara. Informed consents were obtained from all test participants.

2.4. Data analysis
The data was processed using computer software and then calculated as a descriptive statistical analysis of the data demographics. Whereas the data obtained from both examinations will be analyzed regarding sensitivity and specificity and its predictive value and likelihood ratio were determined. All analyses were conducted with SPSS software (version 17.0).

3. Results
The majority of cases involve children aged 5-14 years. Meanwhile, male (53%) were slightly more prevalent than those offemale (47%).

| Age            | Male | Female | RDT N | %  | Male | Female | Microscopic N | %  |
|----------------|------|--------|-------|----|------|--------|---------------|----|
| < 5 years old  | 4 (50)| 4 (50) | 8     | 10.1| 3 (42.9)| 4 (57.1)| 7 | 9.9 |
| 5-14 years old | 34 (68)| 16 (32)| 50    | 63.6| 29 (65.9)| 15 (34.1)| 45 | 62 |
| Age Group       | N   | Fever | Documented fever within 48 hours | Total |
|-----------------|-----|-------|---------------------------------|-------|
| 15-20 years old | 43  | 25    | 19                              | 71    |
| Total           | 80  | 20    | 100                             |       |

Table 2 shows that the symptoms of fever found in malaria patients account for 69% and those with documented fever within 48 hours constitute 2%. While symptoms of fever found in non-malaria patients were 11% and those with documented fever within 48 hours were 18%.

**Table 2. Clinical symptoms of fever on microscopic examination.**

| Microscopic Diagnosis | Clinical Symptoms | Total |
|-----------------------|-------------------|-------|
|                       | Fever             |       |
| Malaria               | 69 (69.0%)        | 71 (71.0%) |
| Non-malaria           | 11 (11.0%)        | 29 (29.0%) |
| Total                 | 80 (80.0%)        | 100 (100%) |
|                       | Documented fever within 48 hours |       |
| Malaria               | 2 (2.0%)          |       |
| Non-malaria           | 18 (18.0%)        |       |
| Total                 | 20 (20.0%)        |       |

Table 3 shows that the mean of parasite density in the group of patients with fever was 2329.13 / mm$^3$ of blood whereas in non-fever patients was 1500 / mm$^3$ of blood. The result of the analysis using Mann Whitney test indicates that there was no significant difference in the mean of parasite density between subjects with fever and with no fever (p = 0.444).

**Table 3. Clinical symptoms by the number of parasitemia.**

| Clinical Symptoms | N   | Parasitemia (/mm$^3$ of blood) | P    |
|-------------------|-----|--------------------------------|------|
| Fever             | 69  | 2329.13 (1417.30)              | 0.444|
| Documented fever within 48 hours | 2  | 1500 (1329.36)                |      |

Table 4 shows that the sensitivity value obtained is 100%, specificity is 72.4%, the positive predictive value (PPV) is 89.9%, the negative predictive value (NPV) is 100%, the likelihood ratio (LR) (+) is 27, 6%, LR (-) is 0% and the prevalence is 71%.
4. Discussion
Based on the result, it is found that the number of malaria patients is more prevalent in males by 53% compared to that of women namely 47% and the majority of cases involved children aged 5-14 years (65%). This result is different from research conducted by Desrinawati in Mandailing Natal (North Sumatra) whereby malaria cases are more common in women namely 58.3% and 41.7% of men.[11] Differences in malaria morbidity rates in male and female or different age groups are in fact determined by several factors namely occupation, level of education, immune system and others.[12-15] This research was conducted on 100 samples in which the results obtained were that as many as 79 subjects had positive RDT while other 71 subjects had positive microscopic. Based on the calculation of the diagnostic test results, the sensitivity obtained was 71/71 (100%), while the specificity was 21/29 (72.4%). The results obtained in this research are generally similar to those conducted in other endemic areas such as those obtained by Arum, et al. at Keruak Health Center, East Lombok Regency, which show 100% sensitivity and 96.99% specificity. [16] However, the results were slightly different from Ginting J et al. on 104 samples in the North Sumatra using Parascreen with sensitivity and specificity of 76.47% and 100%, respectively.[17] In comparison to microscopic examination, diagnosing malaria using RDT is more advantageous in that it can be performed more rapidly in around 3-8 minutes while microscopic examination would take 15-16 minutes.[14] However, unlike microscopic examination, RDT cannot be used to get information about the parasite density in blood. This drawback can be mitigated by following the instructions for storing and using the tool by with the recommendations. Furthermore, classifying patients by clinical symptoms may also assist in increasing the sensitivity of RDT.[19] The results of this research indicate that the Parascreen has a quite high sensitivity and specificity.

5. Conclusion
The results of this research indicate that Parascreen can be used as an alternative diagnostic method for patients with clinical malaria in Lima Puluh District, Batubara Regency especially in areas with a
shortage of skilled medical laboratory workers. However, it cannot be used yet as a substitute for microscopic examination as a gold standard of malaria examination because Parascreen also has a limitation in that it cannot distinguish between two causes of falciparum malaria and vivax malaria.

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