The Pennsylvania State University

The Graduate School

College of Medicine

EVALUATION OF AN AUTOMATED MALARIA PARASITE BLOOD SMEAR READING DEVICE

A Thesis in

Public Health Sciences

by

Azah Abdalla Mohamed

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Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Master of Science
December 2011

The thesis of Azah Abdalla Mohamed was reviewed and approved* by the following:

Vern M .Chinchilli
Distinguish professor and chair Department of Public Health Sciences
Thesis Advisor

John Richie
Professor of Health Evaluation Sciences & Pharmacology

Michele L Shaffer
Associate Professor, Division of Biostatistics and Bioinformatics
Head of the Department of Department or Graduate Program

*Signatures are on file in the Graduate School

ABSTRACT

Accurate identification and quantification of malaria parasites in a timely manner are critical in measuring treatment outcomes. Microscopy is widely used as the "gold standard" method for detecting and quantifying malaria species. However, the method is time consuming. Automated devices have been developed, such as that developed by World Health Technologies (WHT) for a malaria parasite blood smear reader, but have not been thoroughly validated. The objective of this research was to evaluate the WHT automated device through comparison with microscopy. A crude assessment of the sensitivity and specificity was based on the presence or absence of malaria parasite using a set of blood smear slides standardized according to the World Health Organization (WHO), (n= 55), which consists of patient derived slides, 20 of which are positive for malaria, 20 are negative for malaria and 15 are plasmodium falciparum positive control slides. In addition 150 blood smear slides of unknown status were available from the Household Survey in Equatorial Guinea. Each slide was analyzed by the microscopy and for all positive slides, the particular species were determined. A square-root transformation of the counts was calculated prior to the comparison of methods. For the WHO slides, the WHT device resulted in 88.6% sensitivity (For the Household Survey slides, sensitivity was 100% (95% CI = 0.75-1.00) and specificity was 94% (95% CI = 0.90-0.99). The findings showed different results regarding the sensitivity and specificity performance of the WHT device between the WHO slides and Household Survey slides, but they are comparable to the performance of humans. Density values for positive slides were significantly higher for the WHT device compared to microscopy.

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ACKNOWLEDGEMENTS

I would like to thank Dr Roy Prescott, PhD, and Dr Robert Jordan from Hydas World Health at Hershey for providing the data and for their support. I thank and appreciate Dr Vern M Chinchilli, PhD Distinguished Professor and Chair of the Department of Public Health Sciences for his support and advice on the analysis.

Introduction:

Malaria is serious vector-born disease caused by genus plasmodium consisting of four species (*Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale,* and *Plasmodium vivax*). Malaria is transmitted to humans through bites of infected female anopheles mosquitoes. According to the World Health Organization malaria causes over one million deaths each year in all age groups, but mostly in young children and pregnant women. It also causes over 300 million clinical cases each year. (WHO, 2010) The detection of the malaria parasite of stained blood smears by the microscope has been the primary and widely used technique to detect the malaria parasite and estimate the parasite density. Therefore, microscopy has been known as a gold-standard technique. However, it is time consuming and dependent on the technicians' experience and judgment when selecting the slide's fields. (O'Meara et al., 2006)

Alternative methods for malaria diagnosis such as Rapid Diagnostic Testing, and Polymerase Chain Reaction (PCR), used to determine the presence of infection, have been shown to be successful with respect to sensitivity for identifying the malaria parasite in previous studies, but they are expensive and not commonly accessible in comparison to the gold-standard method. Each of these techniques suffers from technical limitations covered later.

Implementation of standardized, accurate, accessible and timely methods of malaria diagnosis is needed. Thus, this study was conducted to evaluate the accuracy of an automated malaria blood smear scanning device developed by World Health Technology (WHT). The objectives of this device are to detect the presence or absence of malaria parasites of blood smear films in terms of positive and negative for the infection, and to identify malaria species in a timely manner. The (WHT) device scans the entire slide to identify malaria species, if present. After scanning, the pictures are input into an algorithm that is an essential recognition pattern of the parasites. So when the device finds such a pattern, it literally circles the images and counts it as a positive for the malaria parasite. Images are digitally saved and can be re-analyzed by the computer or checked by technicians at anytime.

Methods:

The evaluation of the WHT device involved a series of data collection efforts and analysis of slides. Details of data collection, slide preparation, and slide selection are summarized below.

Analysis:

First, a crude assessment of the sensitivity and specificity was determined based on the presence or absence of the malaria parasite, and their 95% confidence intervals were constructed using exact binomial methods. Second analysis of square-root transformations of the counts of all positive slides was performed based on the expert readers, with 99% confidence intervals constructed on the square-root scale and then back-transformed to the original scale. All calculations were performed using SAS 9.2 statistical software.

Data collection:

a) 55 "WHO" Slides:

Fifty-five slides were obtained from Hydas World Health, standardized according to the World Health Organization (WHO), of which the true diagnosis was known. They were used as a "standard test" to compare results after being read on the device. Twenty slides were positive, and twenty slides were negative. These 40 slides were used to assess the device's ability to detect the presence or absence of malaria parasite and the ability to identify the species. Additionally, there were 15 "counting" slides (plasmodium falciparum positive), which were used to assess the ability to estimate the parasite density

b) EGHS slides

Blood was collected from 150 unknown status donors during the Household Survey in Equatorial Guinea. A database was created to contain the donor's age, gender, sentinel site source, and the hemoglobin. Each subject's blood was also tested by the Rapid Diagnostic Testing (RDT) method for the purpose of the survey. One hundred and fifty slides were prepared according to a standardized technique. The set of the 150 slides

was divided into six boxes of 25 slides each, and sent to six experts. When a box was read, it was returned and sent to the next person to establish the true value. Reported results included number of parasites, species identification (*Plasmodium falciparum, Plasmodium ovale, Plasmodium malariae, Plasmodium vivax*, or mix), white blood cell (WBC) and red blood cell (RBC) counts if used, and remarks about the quality of the smear.

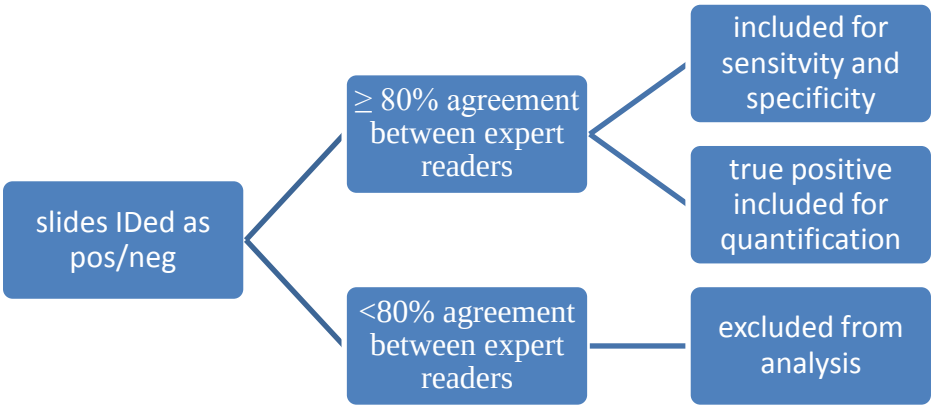
Slide preparation:

Thick films and thins films slides were made from the same blood donor and smeared within an hour of collection to maintain the leukocytes and parasite morphology. A precise micropipette 6µl of blood was transferred into clean 76x26mm slides. Using a corner of another slide, blood was evenly swirled. A thick smear enables red blood cells to lyse, which is a more likely determinant for the presence and identification of the parasite, but is does not differentiate the species. Then 2µl blood was transferred to a clean slide, using the edge of another slide to spread the blood in order to create feathered-edge films. Thin films were fixed with methanol to maintain the red cells morphology. Both films were air-dried over night and then stained in a 3% solution of Giemsa stain.(*malaria microscopy quality assurance manual*).

Slide selection:

Expert readers were asked to use the guidelines for slide reading methodology. On thick films when the expert counted the WBCs, the parasite density estimate was reported by the number of WBCs multiplied by a standard multiplier of 8,000WBC/µl. if the expert used the RBC's counting method, then the parasite density was multiplied by 4,500,000 which was equal to the percent of parasite in red blood cells (Maguire et al., 2006) . Eight slides were reported broken slides. One hundred and six slides were identified negative for malaria parasite by at experts. Thirteen slides were positive for malaria (pf positive). Twenty-three slides were excluded. Exclusion and inclusion criteria for the slides were established when the experts showed variation on agreement or disagreement of the presence or identification of the parasite. As shown in Figure 1.

Figure 1: Exclusion and inclusion criteria



Results:

In assessing the ability of the device to read all the positive slides for malaria infection as having malaria species (sensitivity), the 20 positive slides and the 15 "counting" slides from the WHO validated slides were used. From the WHO dataset, 20 positive slides and the 15 positive slides for falciparum, the device identified 31 slides as positive and missed 4 slides. In assessing the ability of the device to read all negative slides as no parasite seen (specificity), 20 negative slides were used. The device identified 14 slides as negative and missed 6 slides. Those values were expressed in terms of true positive, false negative, true negative, and false positive respectively. The sensitivity and specificity were calculated along with their 95% confidence intervals and the results are summarized in Tables 1 and 2.

Table 1:Exact and asympototic 95% confidence intravals for sensitivity

The FREQ Procedure

machine	Frequency	Percent	Cumulative Frequency	Cumulative Percent
Positive	31	88.57	31	88.57
Negative	4	11.43	35	100.00

Binomial Proportion for machine = Positive

Proportion	0.8857
ASE	0.0538
95% Lower Conf Limit	0.7803
95% Upper Conf Limit	0.9911
Exact Conf Limits	
95% Lower Conf Limit	0.7326
95% Upper Conf Limit	0.9680
Test of H0: Proportion = 0.5	
ASE under H0	0.0845
Z	4.5638
One-sided Pr > Z	<.0001
Two-sided Pr > |Z|	<.0001

Sample Size = 35

Table 2: Exact and asymptotic 95% confidence intervals for specificity

The FREQ Procedure

machine	Frequency	Percent	Cumulative Frequency	Cumulative Percent
Negative	14	70.00	14	70.00
Positive	6	30.00	20	100.00

Binomial Proportion for machine = Negative

Proportion	0.7000
ASE	0.1025
95% Lower Conf Limit	0.4992
95% Upper Conf Limit	0.9008
Exact Conf Limits	
95% Lower Conf Limit	0.4572
95% Upper Conf Limit	0.8811
Test of H0: Proportion = 0.5	
ASE under H0	0.1118
Z	1.7889
One-sided Pr > Z	0.0368
Two-sided Pr > |Z|	0.0736

Sample Size = 20

Using SAS the square-root transformation for the 15"counting" slides was calculated. Twenty-five percent below and above the sample median are accepted limits according to the WHO. We also calculated the 99% CI. Only one of the 15 slides as highlighted in Table 3 was closer to the established limits.

Table (3)

	Machine	MEDIAN	WHO std 25% below	WHO std 25% above	Confidence Interval 99% Lower Limit	Interval 99% upper limit
Count slide 1	5,320	340	255	425	239	850
Count slide 2	3,492	1,048	786	1,310	742	1,227
Count slide 3	4,480	1,321	991	1,651	855	1,984
Count slide 4	2,175	1,40	1,053	1,754	1,001	1,610
Count slide 5	3,081	1,404	1,053	1,754	1,001	1,610
Count slide 6	7,042	2,625	1,969	3,281	1,936	2,956
Count slide 7	1,608	2,261	1,696	2,827	1,756	2,795
Count slide 8	250,000	165,500	124,125	206,875	123,455	235,865
Count slide 9	2,100	1,620	1,215	2,024	1,323	2,031
Count slide10	5,072	340	255	425	239	850
Count slide11	3,096	1,048	786	1,310	742	1,227
Count slide12	204	129	97	161	92	174
Count slide13	192	154	116	193	79	199
Count slide14	8,880	1,321	991	1,651	855	1,984
Count slide15	412	1,620	1,215	2,024	1,323	2,031

Results obtained by the WHT on the Household Survey slides are summarized in Table 4, and calculated based on the following formulas:

Sensitivity = true positive/ (true positive+false negative) = 13/13 = 1.00 with 95% confidence interval = (0.75, 1.00)

Specificity = true negative/ (true negative+ false positive) = 100/106 = 0.94 with 95% confidence interval = (0.90, 0.99).

Table 4:

			reader	
		negative		positive
AHT	Negative	100		0
	Positive	6		13

A transformation for the true positive slides that were read by WHT/AHT device, the mean, the 99% lower confidence interval and upper confidence interval were calculated as seen in Table 5. As highlighted in five slides the device read the parasite density within the lower and upper limits.

Table 5:

SLIDE #	WHT COUNT	COUNT LCI	MEAN	COUNT UCI
44	100	247	930	2050
56	800	429	705	1049
61	2800	419	681	1006
63	100	22	53	96
68	8000	1056	1974	3176
72	1600	156	548	1178
74	150	47	98	168
78	50	24	124	301
115	31600	24025	34869	47726
117	17200	38770	55681	75645
118	571	392	751	1225
137	1500	305	728	1328
143	10000	125	171	226

Discussion:

Microscopy examination of thin and thick blood smear films remains the gold standard in detecting, identifying and quantifying malaria species. Despite the accuracy and sensitivity of the method, limitations exist. The limitation of microscopy and other malaria diagnosis methods are identified in Figure 2. A number of studies previously recommended resolving and overcoming the problems with the microscopy method by developing a digital image system and automating the examination of blood smears (Ma, Harrison, Wang, & Coppel, 2010; Ross, Pritchard, Rubin, & Duse, 2006). Published studies suggested that irregular distribution of the parasites density in the blood results in discrepancies.(O'Meara, et al., 2006) . Another observation from previous studies was that abnormal distributions of WBCs also lead to significant discrepancies. The quality and handling of the smear is one of the factors of discrepancy as indicated in Figure 3 observations and remarks from the experts about the quality and contamination of the smear. This study method demonstrated the accuracy and the ability of the World Health Technology device in detecting the presence or absence of malaria species in term of sensitivity and specificity. Sensitivity of (88.57%) and (70%) specificity (from WHO slides), and sensitivity (100%) and (94%) specificity (from the Household Survey slides) obtained by the WHT device are significant and consistent with other studies (Maguire, et al., 2006). Alexander et al. (2010) defined a method to increase the acceptable limits of agreement by using the square-root transformation as a way to provide normal confidence intervals on the original scale of the parasite density (parasite/µl).(Alexander et al., 2010) In this study method the researchers transformed all the positive counts slides and calculated the 99% CI to provide the best range of acceptable quantification in variability with parasite density. However, the quality of the performance of the WHT device in counting the parasite density compared to the microscopy still tended to result in higher counts. It could be one of the weaknesses of the WHT device in differentiating between artifacts and parasite other than malaria, thereby counting them as malaria parasite. The WHT device, as a timely, simple methodology, resulted in significant presentation on sensitivity and

specificity. However, based on the results, the WHT device is substantially over-counting malaria parasite. From a clinical perspective, those overestimations of parasite density may lead to overestimation of malaria morbidity and misperceptions of therapeutic and treatment application, which may be considered a weakness needing further investigation in order to improve the quality of the device.

Contamination

Figure 2: Some limitations associated with microscopy and others diagnosis methods

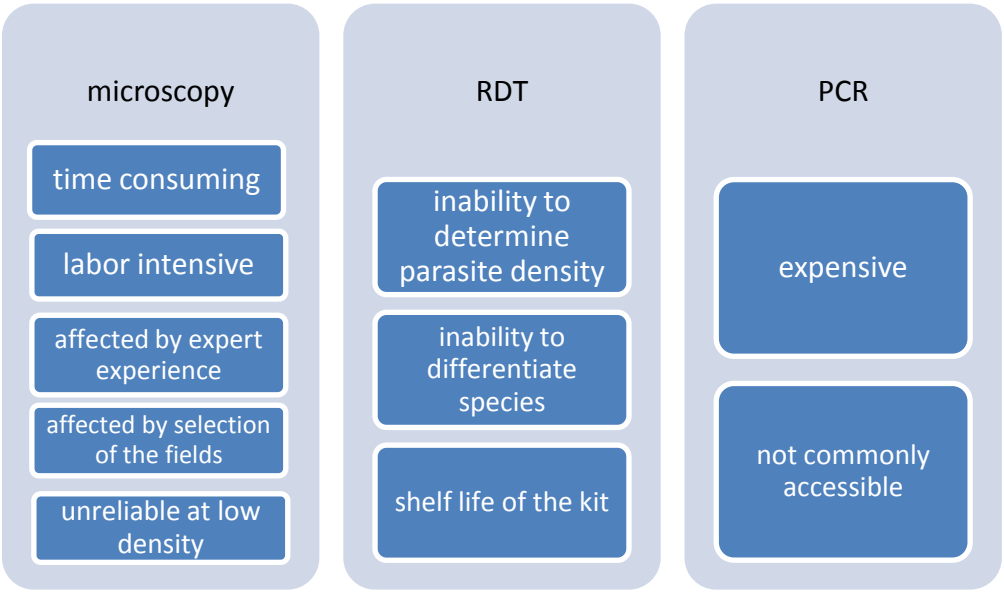
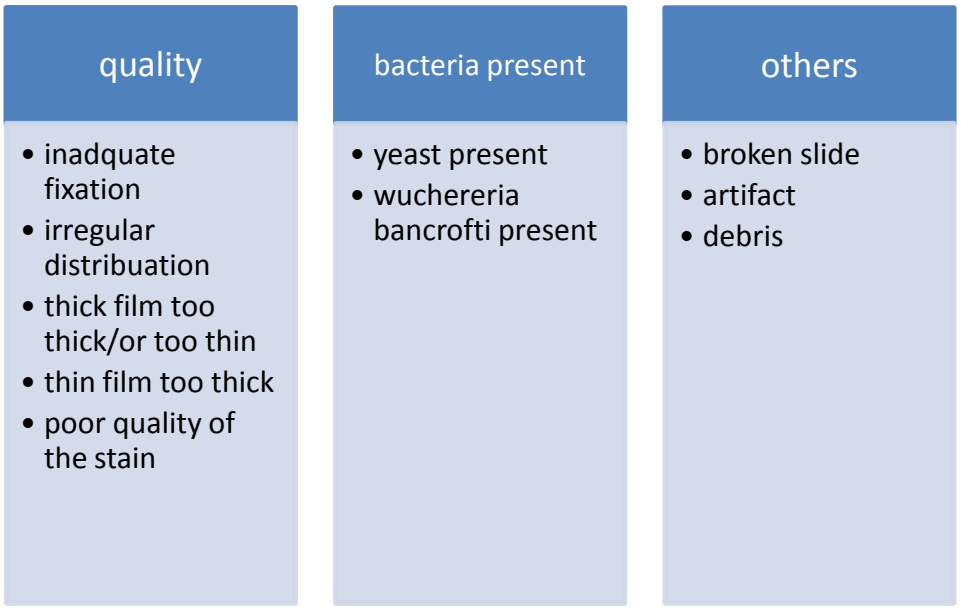


Figure 3: Comments about the slides and smear quality observed by the experts



Conclusion:

Automated image analysis, such as the WHT device, can be considered sensitive in diagnosis of malaria parasite in a timely manner, as results obtained in this research showed. However, improvement is needed in quantification of malaria density in order for the WHT device to serve as an alternative to the microscopy.

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