NOTE

The study CL-IN002 was collaborative study carried out by National Institute of Malaria Research (ICMR), Sector 8, Dwarka, New Delhi, India and Parasight Ltd., Jerusalem Technology Garden, Jerusalem 96951, Israel. NIMR team carried out malaria microscopy and Polymerase Chain Reaction assays and Parasight team carried out testing of the blinded samples with device. This report has been jointly prepared by the undersigned.

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Efficacy of the Parasight P1 device for malaria diagnosis

CL-IN002

Clinical Trial Report

August 8, 2013

Study Sponsor:

Parasight Ltd
Jerusalem Technology Park
Israel

Study carried out by:

National Institute of Malaria Research
Sector 8 Dwarka, New Delhi
India

1 General Information

1.1 Protocol Details

Efficacy of the Parasight P1 device for malaria diagnostics
CL- IN002
July, 2012 to June 2013

1.2 Study sponsor

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1.5 Study location

National Institute of Malaria Research, New Delhi
and
Wenlock Hospital, Mangalore

2 Background:

According to the World Health Organization there were around 216M cases of malaria in 106 malaria-endemic countries with an estimated figure of 655,000 deaths in 2010. Half of the Earth population is at risk of being infected by malaria.

Definitive diagnosis of malaria is imperative for rapid cure, reducing morbidity and mortality, preventing unnecessary use of antimalarials and thus delaying drug resistance and also preventing side effects. As a result, the WHO now recommends against empiric therapy, stating that all cases of suspected malaria should be

confirmed using malaria diagnostic tests prior to treatment. Currently the diagnosis of malaria relies on manual microscopy, immunologic techniques, and Polymerase Chain Reaction (PCR). However, these technologies are lacking: microscopy is time- and labor- intensive and requires qualified personnel, and has issue of accessibility, and is subjective; the Rapid Diagnostic Tests suffer from issues like transport and storage, persistence of hrp2 in blood, inability to detect very low parasitaemias, inability to count the parasites etc. PCR has potential to detect low parasitaemia but is expensive, time consuming, and requires highly trained personnel. The Parasight P1 Device aims to overcome these deficits: the computer-vision-based technology is designed for fast, accurate and cost effective diagnosis of malaria in blood samples. Most importantly, it can also report parasitemia levels.

3 Current diagnostic methods:

Microscopic examination of blood smears is considered to be the "gold standard" for malaria diagnosis. Blood samples are prepared on microscope slides in the form of thick or thin smears, dried, stained, and examined using benchtop microscopes. The technique requires significant time from a trained technician. This limits throughput, raises costs, and creates significant inter-user variability.

Immunologic methods such as Rapid Diagnostic Tests (RDTs) for malaria are currently favored in clinical practice wherever microscopy equipment or suitably trained technicians are in short supply. Though RDTs are very useful in field settings, they have quality issues arising due to storage/transport, antigen type/variation, use by health workers, etc.

PCR tests detect pathogens on the basis of their distinctive genetic information (DNA or RNA). PCR tests for parasites are both very sensitive and very specific, and consequently they are often used to confirm positive diagnoses and species determination when the result is critical. However, these tests:

- Are expensive ($15-50)
- Are time consuming, taking 12 hours to perform and typically having at least a 24-hour turnaround

- Require sterile conditions and a designated isolated room, and run a risk for contamination
- Require a high level of expertise and thus are only available at specialized laboratories and academic medical centers
- Can only detect one parasite species per test, and can be too specific, missing unexpected strains or genetic polymorphism

4 The Parasight P1 Device

Parasight has developed a diagnostic device technology for analysis of blood anomalies using computer vision. The algorithms of the P1 device detect the visual differences between healthy and parasite infected blood cells with high precision even at low concentration of parasites. The sample preparation method of the device is based on custom-built cartridges that facilitate the formation of a monolayer of blood cells within seconds, concurrently staining the sample. This method creates a time-efficient, standardized and uniform version of a "thin blood smear". The cartridge is loaded into this automated device, which rapidly scans, captures, and analyzes a large number of high-resolution images of blood samples. Each microscopic field is captured under several imaging channels specially developed to integrate with Parasight's patent pending sample preparation method. The images are processed using machine-vision techniques to detect anomalies. Total processing time is 3-5 minutes per sample. Upon the detection of anomaly or pathogen the device's simple user-interface provides the diagnosis, including number of parasites per microliter and the species.

5 Methodology

This trial aimed to evaluate the efficacy of the device, as measured by the sensitivity and specificity at different levels of parasitemia for the four common species of malaria. The trial was conducted with **431** consented patients, 43% over original protocol targets, which were: 100 positive (including at least 30 samples of *P. falciparum*, and 30 *of P. vivax*) and 200 negative consenting participants. Sensitivity and specificity of the device were compared against PCR, and conventional

microscopy. Rapid Diagnostic Tests were used for initial patient screening. All patients were symptomatic.

5.1 Endpoints

- Primary
    - Assess P1 specificity and sensitivity in diagnosing malaria

      Target: 98% specificity; 98% sensitivity

      Reference: PCR
- Secondary
    - Limit of detection in terms of parasitemia (reflects on primary)
    - Accuracy of species identification (Pf/Pv)
    - Accuracy of parasitemia estimation
    - Compare P1 to manual microscopy

5.2 Study design

This was a single center, prospective, non randomized, blinded trial. All technicians were blinded to the results of each branch of the study. Comparison between study branches was done only after data collection and the examination was complete.

5.3 Study Procedures

SUBJECT RECRUITMENT AND ENROLLMENT: All patients presenting with fever at the NIMR clinic were considered eligible for enrollment and were given the option to participate. Informed consent was obtained prior to sample collection.

SAMPLE COLLECTION: Suspected patients for *P. vivax* and *P. falciparum* were diagnosed in the routine way in the malaria clinic of Wenlock Hospital, Mangalore, using Giemsa staining followed by microscopic examination (and/or malaria RDTs as routinely used in the clinic). Malaria assessment was solely based on the Wenlock malaria clinic diagnosis process and patients' course of treatment (as per National Policy) was not changed due to the Parasite P1 device or by the NIMR microscopy

diagnoses. In parallel, at least 4 µL of blood in EDTA was collected in microfuge tube. These samples were scanned onsite using the P1 device while P1 final analysis was conducted blindly in Parasight's [redacted] facility in Israel. In addition, blood was also collected as filter paper spots for PCR evaluation at NIMR, and was used for the preparation of two Giemsa stained smears for microscopic examination at NIMR. The Giemsa stained smears and filter papers were transported to NIMR, New Delhi.

ANALYSIS: the Giemsa slides were reviewed by a NIMR microscopist at Wenlock NIMR clinic in Mangalore, and by a second microscopist at the NIMR laboratory in New-Delhi. If there was disagreement between the two reads, or a discrepancy in parasitemia level of greater than 50%, a third microscopist reviewed the slide and made a final determination. PCR assays were carried out at NIMR.

The Parasight digital imaging scanning was carried out onsite. Blood droplet was stained and diluted in a proprietary solution. This diluted sample was placed inside a flow-cell disposable slide. The slide was then loaded into the P1 machine where it was scanned automatically. Each field was autofocused and then scanned with three different colored illumination sources to highlight different stains. The machine scanned a total of 270 fields @ 20X magnification (equivalent to 6750 fields @ 100X), corresponding to 0.2ul of blood. The complete scan took less than 5 minutes per sample. At peak participant volume in CL-1N002, 56 patients were analyzed in an 8hr working day.

Computer vision and statistical models were used to detect the malaria parasites. The algorithm uses fluorescent cues to detect RNA and DNA hotspots and then classifies these into white blood cells, parasites, or "other". The algorithm also estimates RBC density. Using statistical models, Parasight P1 determined infection status, parasitemia levels, and species. Diagnosis and parasitemia are statistical constructs, and there is an *Internal Calibration Parameter* that determines whether a sample is considered "negative" that can be adjusted to optimize the tradeoff between sensitivity and specificity for the use at hand (eg screening or confirmation).

6 Analysis

6.1 Statistical Methods

Sensitivity and Specificity analysis and 95% Confidence Intervals (CIs) were computed using a 2x2 table for outcomes of the tested device and the reference outcome.

Test sensitivity (conditional probability that the test is positive if the condition is positive), calculated by the following formula:

Sensitivity = (True Positive) / (True Positive + False Negative) X 100

Test specificity (conditional probability that the test is normal if the condition is normal (negative), calculated by the following formula:

Specificity = (True Negative) / (True Negative + False Positive) X 100

Kappa Coefficients were calculated for analyzing the agreement between the diagnoses of the two microscopists.

The data was analyzed using the SAS ® version 9.1 (SAS Institute, Cary North Carolina).

6.2 Rejected samples

Of 431 patients consented to participate, 67 were excluded from the study for various reasons:

- Operator errors, such as slide placement error, insufficient filling or overfilling of the slide.
- External technical problems, such as interruptions during running of the test and power failure.
- Missing PCR results.
- Samples marked as undecided by the automated algorithm.

  As part of the automated computer analysis, certain samples are flagged as suspicious or problematic. Since the underlying reason for some of the observed abnormalities is not always clear, some such samples are

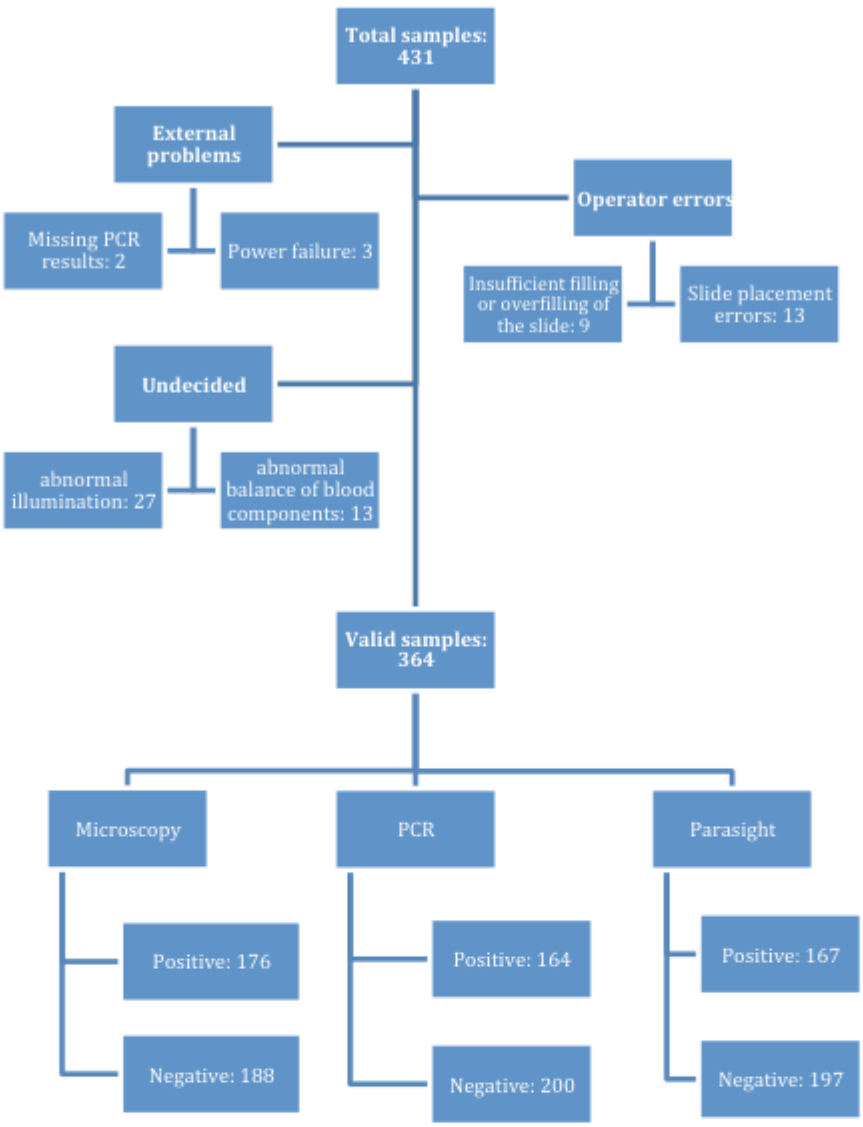
classified as “undecided” so as to not mislead the user with an erroneous positive or negative result.

Category	Number rejected
Operator errors	22
External technical problems	3
Missing PCR results	2
Samples marked as undecided by the automated algorithm due to abnormal illumination or brightness	27
Samples marked as undecided by the automated algorithm due to abnormal balance of blood cell components	13

6.3 Criteria for results reporting and inconsistencies

We elected to have two microscopists review each slide, with a third, senior microscopist’s evaluation in cases of discordance. 23 samples required third microscopist review.

PCR assessment was repeated in cases where the PCR results were inconsistent with microscopy. To reduce the risk of potential cross-contaminations, mislabeling, or other errors, DNA samples were scraped from the Giemsa slides. In 37 cases out of the 48 PCR-microscopy inconsistency cases, inconsistencies were resolved in repeated PCR assessment.



7 Results

7.1 Sensitivity and Specificity

The study's population involved 364 samples that were evaluated by 3 different methods:

- Parasight device
- PCR
- Microscopy

Sensitivity and Specificity rates were calculated between Parasight and PCR, and between Parasight and microscopy.

7.2 Parasight vs PCR

As part of the software analysis, a specific parameter is calibrated in order to decide whether a sample is infected. One of the aims of the study was to determine the *Internal Calibration Parameter* that optimizes the balance between sensitivity and specificity.

The *Internal Calibration Parameter* used in the study for the primary objective was set to 3. Our data analysis suggests that a more suitable *Internal Calibration Parameter* value is 6. With this setting, the device's sensitivity and specificity, when compared to PCR, were found to be **99.4%** (95%CI 96.6%-99.9%) and **98.0%** (95%CI 95.0%-99.2%) respectively.

The following table summarizes the sensitivity of Parasight compared to PCR at different parasitemia ranges, when using an *Internal Calibration Parameter* value of 6 (the parasitemia of a sample is determined by the microscopy reference):

Sensitivity of P1 device vs. PCR calculated for different Parasitemia Ranges

	Sensitivity		
Parasitemia range (p/uL)	**Percent**	**in numbers**	**95% CI**
100 – 200	50%	1/2	9.5% - 91%
200 – 500	100%	7/7	72% - 100%
500 – 1000	100%	14/14	83% - 100%
> 1000	100%	141/141	98.1% - 100%
Overall	99.4%	163/164	96.6% - 99.9%

Specificity of P1 device vs. PCR

Specificity		
Percent	**in numbers**	**95% CI**
98%	196/200	95.0% - 99.2%

7.3 Parasight vs Microscopy

Sensitivity and specificity of Parasight compared to microscopy were found to be 93.75% and 95.0%, respectively.

Sensitivity and Specificity of P1 device vs. Microscopy

Sens.			**Spec.**		
percent	**in numbers**	**95% CI**	**percent**	**in numbers**	**95% CI**
93.75%	165/176	89.2% - 96.5%	95.00%	171/180	90.8% - 97.3%

7.4 Speciation

Species identification is important for clinicians in order to determine the correct treatment for a patient (medication, dosage, and duration of treatment).

The ability of the device to identify two types of species (Pv = *Plasmodium vivax* and Pf = *Plasmodium falciparum*) was compared with PCR. The current version of the device did not support mixed infection reporting. Amongst samples found to be positive by both PCR and Parasight, Parasight speciated 78.6% of PCR Pf+ samples and 96.9% of PCR Pv+ samples, as shown in the table below:

Speciation accuracy divided according to treatment groups

Treatment POV	Parasight accuracy	in numbers	95% CI
[redacted]. Treatment	**97.0%**	**96/99**	**91.5% - 99%**
[redacted]. Treatment	78.57%	33/42	64.1% - 88.3%
Mix infection Treatment[5]	0%	0 / 13	NA

[5] **All of the mixed infection samples were positively detected by Parasight as infected. 11 mixed infections were reported as Pv, and the other 2 were reported as Pf.**

7.5 Parasitemia

Parasight parasitemia read outs have never been calibrated to microscopy and the relationship is non-linear at different parasitemia levels. Spearman's rank correlation coefficient analysis is preferred for monotonic relationships. The Spearman's rank correlation coefficient calculated when comparing Parasight's parasitemia reporting to microscopy on infected samples is as follows:

Spearman's correlation coefficients, N = 164, Prob> |r| under H0: Rho=0

	parasight_parasitemia
micmean_parasitemia	0.86 (1)
Parasitemia by microscopy[6]	<.0001 (2)

Following the World Health Organization criteria, Parasight was analyzed on whether or not it produced parasitemia levels of +/- 50% of microscopy. Microscopy parasitemia was considered as the average of the two microscopists' estimation, or the third microscopist's estimation in cases of disagreement. Parasight was within 50% in 71.3% of cases (in 117 out of 164).

7.6 Conclusion/Discussion

The study evaluates a novel platform for diagnosing malaria. It showed sensitivity of 99.4% and specificity of 98.0% when compared to PCR. Moreover, the study showed that the platform also provides high sensitivity in cases of low parasitemia.

With regards to speciation, Parasight is quite accurate at determining the species of the parasite, but the tested version of the device did not identify mixed infections. With regards to parasitemia, Parasight correlates quite well with the assessment of microscopists.

7.7 Limitations

Though the device showed good sensitivity and specificity against PCR and microscopy, few questions still remain to be answered. The software version used in this study is not equipped to detect mixed infections, and while all the mixed infection samples were diagnosed as infected by the device, it interpreted them as *P. vivax*.

The device is capable of identifying the infection stage of a sample, but verifying the accuracy of infection stage identification was not in the scope of this study.

- There were operator errors responsible for indeterminate results. Such errors could be eliminated by including a modified sample-loading mechanism and improved sample-preparation procedures. Furthermore, operator errors can be identified during the scanning of the sample, in which case the user can be notified to re-run the sample.
- There were external conditions that caused test failures. The device could be improved to identify that an interruption has occurred and request the user to re-run the sample.

The tested device did not identify the mixed infections. Hence there is scope for improving the software.

Some samples could not be analyzed due to the reasons listed earlier. However, several software and hardware correction steps were implemented since the data collection completion, aiming at dramatically lowering the rejection and undetermined sample rate. These steps include sample and slide preparation steps to

reduce overfilling or insufficient slide filling and to improve staining solution consistency; slide holding improvements to reduce slide misplacement; and software improvements in handling sample deviation and support real-time user re-scan report flag.

Among the 67 rejected samples, reference results were available for 64 samples. Three were positive for *P. falciparum*, 11 were *P. vivax* and one was mixed infection. Since the device did not provide results for these samples, they could not be included in the analysis.

8 Future work:

CL-IN002 was a single site clinical trial. Multisite studies will help further validating the device.

9 NIMR team

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10 Parasight team

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