A Biochemical Assay Provides Better Diagnosis for Malaria Infection

Ifeanyichukwu Okeke[1*]

[1]*Afinity Laboratories and Biosciences Concerns, Nigeria.*

[*]**Corresponding author**
Ifeanyichukwu Okeke, Afinity Laboratories and Biosciences Concerns, Nigeria.

Abstract
Malaria is a disease caused by Plasmodium parasite and it is the deadliest parasitic disease affecting humanity. Detection of this microscopic organism using currently available techniques in the face of dwindling quality of testing reagents is increasingly becoming quite challenging especially in low prevalent settings and low-density infections. The absence of a particular detection method devoid of shortcomings and pitfalls of significant order prompted the development of a simple biochemical in vitro diagnostic technique for Plasmodium infection detection known as AfinUrimal. In this study the efficacy of AfinUrimal in detecting active malaria infection using stained blood microscopy method as the reference was examined on 397 patients with febrile illness in Jos, Nigeria in the year 2022. Sensitivity/specificity of AfinUrimal method against stained blood microscopy method was 99.42/92.0% respectively. The analysis showed that biochemical detection of malaria infection using AfinUrimal provides greater sensitivity and specificity useful for diagnostic, epidemiological and research purposes.

Keywords: AfinUrimal, Biochemical detection of malaria, Malaria pigment, Hemozoin.

Introduction

Jos is the capital city of Plateau state Nigeria. Plateau state is located in the middle belt region of Nigeria in West Africa and is considered a malaria endemic area with year-round transmission of the parasite which peaks around the mid-months (May-August) of the year. The need for prompt diagnosis to bring about prompt treatment needed to prevent morbidity/mortality arising from malaria infections prompted the development of an easy to use, non-invasive, painless, bloodless, affordable and convenient test (AfinUrimal) for detection of Plasmodial infection. AfinUrimal is a novel biochemical urine malaria test to detect malaria infection using urine instead of blood. Flexibility enhanced in AfinUrimal enables rapid malaria testing, not only at the points of care and conventional laboratories, but also encourage testing anywhere and at anytime yet obtain valid result that will direct informed therapeutic care, (Armstrong-Shellenberg et al., 1987). Presently and predominantly in highly endemic areas, presumptive diagnosis based on clinical observation has been the basis for therapeutic management of patients suspected of having malaria infection, (Moody(a), 2002), and this has been proven to be unreliable and contributes to malaria over-diagnosis resulting in wastage of anti

malaria drugs on patients who do not need them, (Kahama-Maro et al., 2011) and extension of morbidity period, creating chances for further transmission from disease carriers.

Stained blood microscopy method is considered the gold standard but requires well-trained, competent malaria microscopists and rigorous maintenance of functional infrastructures plus effective quality control and quality assurance (Wongsrichanalai et al., 2007). The RDTs that detect malaria antigens such as Plasmodium falciparum histidine-rich protein 2 (PfHRP2), Plasmodium aldolase (PALD), Plasmodium lactate dehydrogenase (PLDH), Plasmodium falciparum hypoxanthine-guanine phosphoribosyl transferase (PfHGPRT) and Plasmodium glutamate dehydrogenase (PGDH) are widely used at the point of care due to portability, (Abdalla et al., 2019) however there are some limitations to the RDTs that have to do with relatively long half-life of the antigens after clearance of the infection (Dalrymple et al,. 2018) and use of blood which not every patient is comfortable with. Polymerase chain reaction method is mostly used for research purposes to detect infections especially at low parasitemia missed by stained blood microscopy and RDT methods, (Lucchi et al,. 2016). In a

study involving another urine malaria test that detects PfHRP2, it was indicated that the test had sensitivity of 83.75% and specificity of 83.48% with turnaround time of 25 minutes though different malaria parasite densities showed different sensitivities as follows: ≤100 parasites /µl of blood 0%, ≤200 parasites /µl of blood 50% and ≥201 parasites /µl of blood 89.71% (Oguonu et al., 2014).

Simple, accurate and timely detection of active malaria infection by utilizing malaria pigment, a metabolic product of the parasite is the basic operation of AfinUrimal. This metabolite is effectively cleared from peripheral blood (macrophages) shortly (72 hours) after clearing the parasite (Kemsner et al., 2009) and much shorter time from plasma. As the quest to have a simple and reliable malaria test continues AfinUrimal was developed with the objectives of providing ultra sensitive, specific, robust, scalable, goodness of use and interpretable result with short turnaround time, all these objectives were met by detecting malaria pigment from urine of exposed and infected persons. A couple of wet chemistry techniques have been used to study malaria pigment in the field of drug screening assay for antimalaria (Ncokazi et al,. 2005) and quantitative determination of phagocytosis of parasitized erythrocytes and of malaria pigment (Schwarzer, Turrini & Arese, 1994) but not much has been seen in the area of malaria diagnosis until the development of AfinUrimal. However, AfinUrimal is not the first test to utilize malaria pigment but it is the first wet chemistry test designed to be deployed for routine laboratory use for patient diagnosis. In their work, Obisesan et al studied on a method that makes use of electrochemical nanosensor to detect the synthetic form of the malaria pigment as malaria biomarker. (Obisesan et al., 2019). In another study, de Melo et al (de Melo et al., 2021) analyzed the performance of a sensitive hemozoin-based malaria diagnostic test (GazelleTM) device that is based on the magneto-optical concept for the detection of paramagnetic hemozoin by depolarizing light without any reagent. Detection can be done by optical methods using dark-field microscopy (Jamjoom, 1983), polarization microscopy (Lawrence & Olson 1996), or flow cytometry (Hanscheid, Valadas & Grobusch, 2000).

As against these methods, AfinUrimal is a biochemical test to detect malaria pigment from urine and has proven to be highly efficient even at a very low parasite density encompassing qualitative, quantitative and semi-quantitative applications. Results can be quantitatively measured on colorimeter. Semi quantitatively results can be matched to a colour chart provided with the test kit. Parasite density has been found to be directly proportional to the optical density. Malaria pigment is produced in increasing amounts by the parasite during the blood stage of its cycle and therefore marks severity and prognostic factor for malaria infection (Hänscheid et al., 2008). Unlike the conventional malaria RDTs which make use of malaria proteins such as Plasmodium falciparum histidine-rich protein 2 (PfHRP2), Plasmodium aldolase (PALD), Plasmodium lactate dehydrogenase (PLDH), Plasmodium falciparum hypoxanthine-guanine phosphoribosyl transferase (PfHGPRT) and Plas-

modium glutamate dehydrogenase (PGDH) which detect malaria from blood, (Ragavan et al., 2018, Krampa et al., 2020 and Jain et al., 2014), AfinUrimal detects malaria pigment from urine. Malaria pigment is an insoluble crystallite that does not provide species-specific diagnosis. It therefore provides universal diagnostic information essential for malaria treatment especially in malaria endemic areas (Chen, Shi & Sullivan, 2001), (Pisciotta et al., 2005, Oliveira et al., 2000 and Coronado, Nadovich & Spadafora, 2014). AfinUrimal test is not affected by hrp2/hrp3 gene deletions that often lead to false negative results.

Abbreviations
LoD: level of detection
OD: optical density
PD: parasite density

Materials and Methods
Study Design
Blood and urine samples from a total of 397 hospital patients of all ages attending Bingham University Teaching Hospital, Jos, Plateau State Nigeria were enrolled during malaria transmission season from May to July, 2022. Those suspected of having malaria were referred for malaria test where blood was used for stained blood microscopy test and the urine samples were treated to AfinUrimal test.

Ethics Statement and Informed Consent
Ethical approval for this study was obtained from Research and Ethics Committee of Bingham University Teaching Hospital (BUTH) Jos, Plateau State Nigeria. All study participants and their parents / guardians were enrolled after giving verbal informed consent in English and Hausa languages for blood specimen and urine sample collection.

Data and Sample Collection
All patients were recruited regardless of body temperature reading. Details taken from the patients included age, sex and hospital registration number. About 2 ml of blood sample was collected into ethylenediamine tetraacetic acid (EDTA) bottle. Thick blood smears were prepared on microscope slide for stained blood microscopy while urine samples were collected into universal container for every patient.

Laboratory Assays
AfinUrimal Test
A measured volume of urine was added to a clean transparent glass test tube. To this was added the AfinUrimal reagent running down through the inner wall to the bottom of the tube. Without mixing a characteristic color immediately develops at the bottom of the tube signifying a reaction between the reagent and malaria pigment. This could be easily seen when the tube is viewed against a white paper placed against a bright environment or white light source. This color is seen within the first five seconds of the test. A solution with a homogenous colour is formed when this is mixed properly and is used to determine the optical density (OD) of the reaction after 15-20 minutes (figure 1).
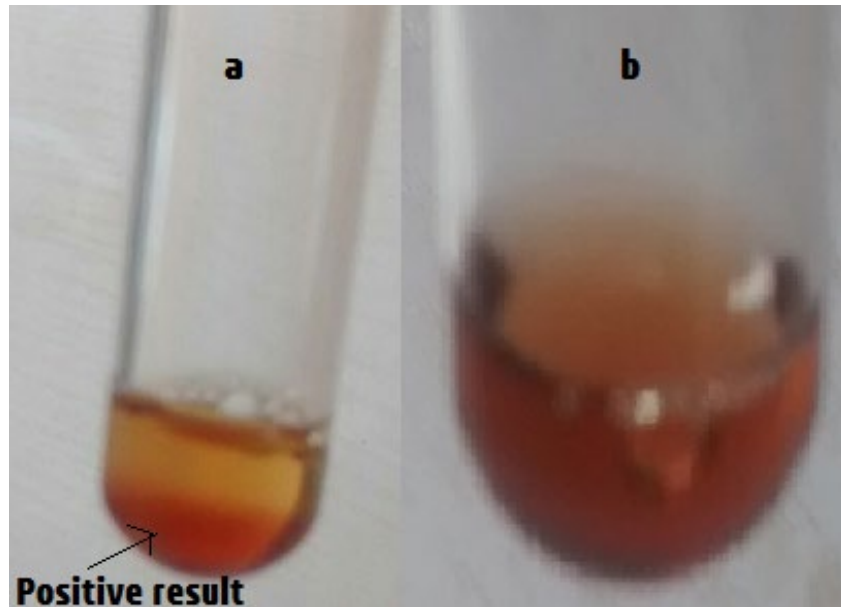
Figure 1: Display of AfinUrimal Test Results by tube method: (a) before mixing, (b) After mixing.
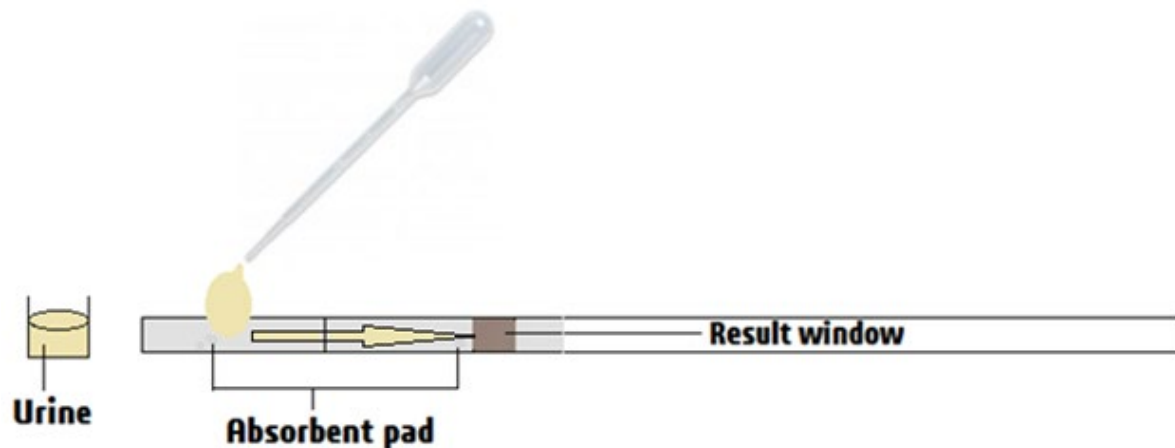

Figure 2: AfinUrimal Testing Procedure.

A proposed modification of this test involves using a microfluidic system for reading and quantification of the reaction from any of the methods is ongoing.

A colour chart provided with the test kit helps to estimate the severity of infection which is proportional to parasite density (PD) and optical density (OD). Optical and parasite densities provide handy quantification of the level of parasitemia (table 1). For the purpose of this study, spectrophotometric reading of the results is matched with microscopy observation as follows: where OD reads $0.09 \leq 0.18$ nm PD is equivalent to $1 \leq 9$ parasites /µl in 100 oil immersion fields of thick blood smear and can be considered to be in 'trace' amount. $0.18 \leq 0.36$ is equivalent to $10 \leq 99$ and can be scored +, $0.36 \leq 0.54$ is equivalent to $100 \leq 999$ for ++, OD of $0.54 \leq 0.72$ is equivalent to $1000 \leq 9999$ for +++, OD $0.72 \leq 0.90$

is equivalent to $10000 \leq 99999$ for ++++ and OD of 0.90 is equivalent to ≥ 100000 parasites /µl of blood and can be scored > ++++. Parasite density is estimated as:

PD=(Number of parasites counted in a given section of the slide)/ (WBC simultaneously counted)xTotal WBC count of participant / µl.   Equation 1.

The + scoring methods as used in this study were purposely to estimate parasite densities based on individual's WBC count /µl. Instances where slides have no parasites in 100 oil immersion fields are scored "Negative" while between 1 and 9 parasites in 100 oil immersion fields of thick blood smear are scored "Trace" (Table 1). However, a WHO publication titled "Basic Malaria Microscopy" Part 1 Learner's guide provides that + is equivalent to 1 to 10 and ++ is equivalent to 11 to 100 parasites per 100 oil immersion

thick film fields. Also that +++ is equivalent 1 to 10 and ++++ is equivalent to more than 10 parasites per single oil immersion field of thick film.

Parasite densities for all participants were estimated using their precise white blood cell (WBC) count. Optical density and parasite density of each sample were used to establish the level of infectivity shown on the color chart (table 1) used in observing the strip. The value of the blank used to zero the instrument was the mean of those that were malaria negative (data not shown), with a range of 0.03-0.13 OD.

Table 1: Color Chart for AfinUrimal Semi Quantitative Analysis.

Color	OD	PD (parasites /µl) in 100 Fields	Score
0.08	≤ 0.09	0	Negative
0.16	0.09≤ 0.18	1 ≤10	Trace
0.28	0.18 ≤ 0.36	10 ≤100	+
0.44	0.36 ≤ 0.54	100 ≤1000	++
0.67	0.54≤ 0.72	1000 ≤ 10000	+++
0.75	0.72 ≤ 0.90	10000 ≤ 100000	++++
0.99	≥ 0.90	100000 ≤ 1000000	>++++

Stained Blood Microscopy

A thick blood smear was made on a clean grease-free microscope slide for the stained blood microscopy method. Giemsa stain was diluted to 10% and the diluted portion was used to stain the thick blood smears for 15 minutes, rinsed in distilled water and dried. The slides were viewed under light microscope at X100 objective and X10 eye piece for presence of malaria parasites and the estimate of parasite density in each slide.

White Blood Cell (WBC) Estimation

For the purpose of estimating the parasite density, total white blood cell was estimated from blood samples for all participants using the improved Neubauer counting chamber. A portion of the thick film where white cells are evenly distributed and parasites well stained is selected for examination and at least 200 WBCs were counted concurrently with all the asexual forms of the parasite in the fields covered, equation 1.

Validation of AfinUrimal

As part of validation activities to assess clinical usefulness and value addition of AfinUrimal test to care of patients. Qualitative and quantitative assessments were carried out to determine the diagnostic validity of the test results from this method. This was ensured so that the result from the test will enable quick and effective decision regarding treatment and provision of preventive measures patients need to take. Interested parties are encouraged to further this study at various scientific dimensions just as obtains with other malaria RDTs.

Data Analysis

The individual's test result using AfinUrimal were classified as true positive (TP), true negative (TN), false positive (FP), false negative (FN) using stained blood microscopy as the reference standard.

In order to determine the sensitivity i.e, the probability that a truly malaria infected individual tests positive by AfinUrimal is given by the equation:

$Sensitivity = (True\ Positive)/(True\ Positive+False\ Negative)$ (Equation 2).

Specificity i.e, the probability that a truly uninfected individual will test negative using AfinUrimal is given by the equation:

$Specificity = (True\ Negative)/(True\ Negative+False\ Positive)$ (Equation 3).

The positive predictive value (PPV) of AfinUrimal was also determined. This tells the probability that those individuals testing positive by AfinUrimal were truly infected by malaria parasite and is given by the equation:

$PPV = (True\ Positive)/(True\ Positive+False\ Positive)$ (Equation 4).

The negative predictive value (NPV) of AfinUrimal which implies that those individuals testing negative by AfinUrimal were actually not infected by malaria parasite and is given by the equation:

$NPV = (True\ Negative)/(True\ Negative+False\ Negative)$ (Equation 5).

Accuracy: the percentage of tests that were correctly detected by AfinUrimal is given by the formula: Items classified correctly/All items classified, or

Accuracy = (True Positive+True Negative)/(Total Number of participants) (Equation 6).

Precision: number of correct positive results/Number of all positive result (*i.e, TP/TP+FP), or, TP/Actual Result.*
Precision = True Positive/True Positive+False Positive (Equation 7).
Error rate: the frequency of errors occurred, defined as "the ratio of total number of data units in error to the total number of data units transmitted". As the error rate increases, the data transmission reliability decreases. It is given by the formula:
Error rate = 1 – Accuracy, or

Error rate =False Positive+False Negative/ Total numberof participants (Equation 8).
In each case, the 95% confidence interval (95% CI) of the respective probabilities was also calculated. (Banoo et al., 2010, Liu et al., 2016).

Results
A total of 397 participants were enrolled into the study. 51.2% male and 48.8% female. Participants below 15 years of age were 13.60%, 15-19 years 23.43%, 20-29 years 30.23%, 30-44 years 16.62% and 45 years and above were 16.12% (table 2). Every study participant had their AfinUrimal alongside stained blood smear microscopy test results. Those testing positive to microscopy and AfinUrimal methods were 346 (87.15%) and 349 (87.91%) respectively (table3).

Table 2: Age Distribution of Study Enrollees.

Age (Years)	Total	%
< 15	54	13.60
15-19	93	23.43
20-29	120	30.23
30-44	66	16.62
45 >	64	16.12
Total	397	100

Table 3: Summary of Results.

	Stained Blood Microscopy N (%)	AfinUrimal N (%)
Positive	346 (87.15)	349 (87.91)
Negative	051 (12.85)	048 (12.09)
Total	397	397

To recognize the performance characteristics of AfinUrimal, the number of true positive (TP) i.e, the number of participants who tested positive when microscopy is positive were 345 (86.90%). False positive (FP) i.e, the number of participants who tested positive when microscopy is testing negative were 4 (1.01%). True negative (TN) is 46 (11.59%) i.e, the number of participants who tested negative when microscopy is testing negative, while false negative (FN) i.e, the number of participants who tested negative when microscopy is testing positive is 2 (0.50%) (table 4).

Table 4: Measure of Diagnostic Parameters.

Justification	AfinUrimal
True positive (TP)	345 (86.90)
False positive (FP)	04 (1.01)
True negative (TN)	46 (11.59)
False negative (FN)	02 (0.50)
Total	397

Findings from the study showed the performance characteristics of AfinUrimal as: sensitivity 99.42% and specificity 92.0%, Positive predictive value (PPV) 98.85% while its negative predictive value (NPV) is 95.83%. The diagnostic accuracy of AfinUrimal was found to be 98.49%, precision 98.85% and error rate 1.51% (table 5, Equations 2-8).

Table 5: Summary of Diagnostic Performance of AfinUrimal.

Sensitivity	Specificity	PPV	NPV	Accuracy	Error rate	Precision
99.42%	92.0%	98.85%	95.83%	98.49%	1.51%.	98.85%

Discussion

This clinic-based study was a deliberate effort to obtain the real-time performance expected of the new diagnostic test for malaria infection with the aim of deploying it for routine use for diagnosis and management of individuals exposed or infected with malaria disease. The high positive rate exhibited by the biochemical detection technique for malaria infection by AfinUrimal showed it has higher sensitivity rate than microscopy method especially at low parasite density. Stained blood microscopy method is more specific than other methods including PCR (Plucinski et al., 2018), especially when carried out by expert malaria microscopist. Observation made from this clinical study on AfinUrimal positions it as a veritable tool for detecting active malaria infection. In a comparative study analysis in DR Congo, sensitivities of available methods were as follows: stained blood film microscopy 76.7%, RDT 86.9%, and PCR 94.6% and specificities of 97.2%, 88.1% and 94.6% in that order (Doctor et al., 2016) where prevalence rate by PCR method was 38.6%. Parasite prevalent among children under 5 years in this area has been put at 0.2% (Agency National de la Statistique at de la Demographine, 2015).

Some limitations are associated with this study: in as much as the sampling population was appropriate for evaluating diagnostic methods, the sampling population were mostly those people residing in the study area which is mostly considered endemic region, and therefore findings cannot be generalized to the overall global population and may differ from those seen in cross-sectional survey among asymptomatic persons and those in low prevalent areas. Two of the samples showed false negative result with AfinUrimal in the presence of high malaria parasite densities (41,150 and 102,724 parasites /µl) with blood stained microscopy method. One of these samples was from a 2-year-old baby who could not produce her urine in the facility while the other sample was run when the reagent was suspected to have faded after a prolonged exposure. A repeat test of the second sample with a fresh reagent returned a positive result. It is important to note that hydration state of patient plays significant role in the concentration of the pigment as poorly hydrated patient with highly concentrated urine tend to yield higher ODs and vice versa.

Application of AfinUrimal as one of the test options available for malaria detection provides user the advantage of conducting a bloodless, painless, non-invasive and rapid test for malaria while achieving high sensitivity, specificity, accuracy and quantitative result with low error rate of 1.51%.

A user can make a choice between the available methods depending on their interest. For instance, high sensitivity of AfinUrimal favours it over stained blood microscopy method for parasite detection with LoD of about <5 parasite /µl of blood based on observation from hemozoin colorimetric assays while the high specificity and differential ability of stained blood microscopy method make it most suitable for research purposes. Stained blood microscopy has sensitivity of 50-500 parasite /µl (Moody(b), 2002). A PCR method has been reported to have LoD of approximately 0.0001 parasites /µl (Romay-Barja et al., 2015) and another reported 2-5 parasites /µl (Pedro et al., 2018).

The use of AfinUrimal with positive predictive value of 98.85% provides that those requiring treatment can be easily identified and treated, key to eliminating malaria since there won't be transmission when there are no parasites [1-31].

Declarations
Competing interests
The authors have no competing interests to declare.

Authors' contributions
Ifeanyichukwu Okeke conceptualized and coordinated the entire study.

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AfinUrimal **UMT** 10mL

URINE MALARIA TEST

INTENDED USE
For detection of malaria pigment in urine.

INTRODUCTION
Malaria pigment is formed by aggregation of the toxic haem groups released from the digestion of haemoglobin of infected erythrocytes into an insoluble non toxic material called hemozoin. This metabolite is effectively cleared from peripheral blood (macrophages) shortly (72 hours) after clearing the parasite and much shorter time from plasma [1] [2]. The AfinUrimal urine malaria test (UMT) is a biochemical method used for the detection of this malaria pigment which is produced by all *Plasmodium species*. Unlike other malaria rapid tests, AfinUrimal test is not affected by *hrp2/hrp3* gene deletions that often lead to false negative results. The AfinUrimal UMT is so named to reflect the laboratory (Afinity Laboratories and Biosciences Concerns) where the product was developed. In the year 2022, Afinity Laboratories Limited redefined malaria diagnosis by way of wet chemistry and requests the World Health Organization (WHO) to repurpose AfinUrimal test as a new protocol for diagnosing and quantifying malaria infection as it is also suitable for epidemiological studies.

PRINCIPLE
Hemozoin in urine undergoes a double replacement reaction with an inorganic ion to produce an organic molecule which further decomposes instantly to liberate metal iron seen as brownish color in the test tube. The amount of hemozoin content of the urine is directly proportional to the amount of metal iron librated.

1. Hemozoin + Inorganic ion __ Organic molecule + Prosthetic group.
2. Prosthetic group __ Fe_2 + Nitric acid.

QUALITY CONTROL
The potency of each batch of AfinUrimal is determined by comparing the result produced by a sample tested with the previous batch and that by the new batch. With proper storage, it is expected that the previous batch produce the same value of result with the new batch of reagent when used on the same sample.

REAGENT STORAGE AND STABILITY
AfinUrimal is stable at room temperature and can be stored at temperatures between 2 to 30 °C. Do not freeze. Do not expose to light. Exposure to light will result in loss of potency. AfinUrimal must always be in its original container.

BIOSAFETY
1. Avoid splashing of reagents. In case of any spill, the contaminated area should be decontaminated immediately using a mild alkaline solution.
2. Any waste material should be disposed of in accordance with local requirements.
3. Do not smoke, eat or drink in the area where kit reagents are handled.

PRECAUTIONS[3]
1. Avoid using discolored urine. Cloudy urine should be allowed to settle or centrifuged at ≤500 g for 5 minutes and the supernatant used.
2. Use only clean dry transparent test tube to run the reaction.
3. Take extreme care to avoid contaminating the reagent with extraneous colors.
4. Avoid direct exposure of reagent to sunlight.

PROCEDURE [1]
AfinUrimal test is performed by pipetting 800μL of urine and 200 μL of AfinUrimal reagent into a clean transparent glass test tube. It is advisable to allow the AfinUrimal reagent to slowly run down the tube through the inner wall to the bottom of the tube. Without mixing a characteristic color immediately develops at the bottom of the tube signifying a reaction between the reagent and malaria pigment. This could be easily seen when the tube is viewed against a white paper placed against a bright environment or white light source. This color is seen within the first five seconds of the test. A solution with a homogenous color is formed when this is gently mixed properly (**do not shake vigorously**) and can be used to determine the optical density (OD) of the reaction using colorimeter[3] or spectrophotometer after 15-20 minutes with wavelength set at 540 nm (figure 1).
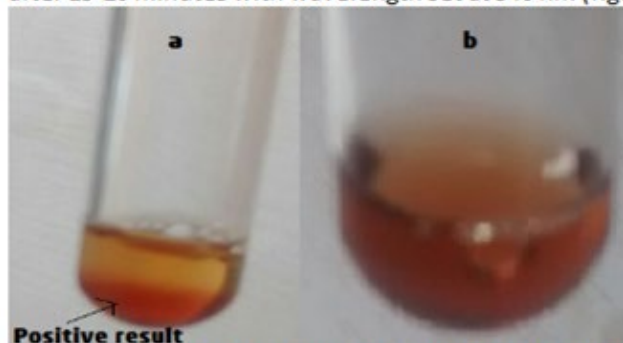

Figure 1: Display of AfinUrimal Test Results: (a) before mixing, (b) After mixing.

INTERPRETATION OF RESULTS[1]

Each AfinUrimal test comes with a result plate in which patient details and result can be entered.

AfinUrimal Result Plate
Novel urine malaria test — Detecting malaria pigment in urine

Pat_Name:_____ Num:_____ Sex:_____ Age:_____

Pat OD	Color	OD	PD (parasites /µl) in 100 Fields	Score
	0.09	≤ 0.09	0	Negative
	0.16	0.09 ≤ 0.18	1 ≤ 10	Trace
	0.28	0.18 ≤ 0.36	10 ≤ 100	+
	0.44	0.36 ≤ 0.54	100 ≤ 1000	+ +
	0.67	0.54 ≤ 0.72	1000 ≤ 10000	+ + +
	0.75	0.72 ≤ 0.90	10000 ≤ 100000	+ + + +
	0.99	≥ 0.90	100000 ≤ 1000000	> + + + +

Key: OD = Optical density, PD = Parasite density

Figure 2: AfinUrimal Result Plate

In the absence of a colorimeter or spectrophotometer, an observer can read off the result right from the test tube by comparing the color with the colors in the chart provided with the test kit (figure 2). The optical density can be used to deduce the parasite density[4].

Note that hydration state of patient plays significant role in the concentration of the pigment as poorly hydrated patient with highly concentrated urine tend to yield higher ODs and vice versa. When optical densities are ≥ 0.90 with scores ≥ ++++, the reaction can be doubly diluted, mixed properly and re-run on colorimeter or spectrophotometer immediately. The scores obtained multiplied by 2 to obtain the actual scores and parasite densities.

MATERIALS PROVIDED
1. A 10 mL vial of AfinUrimal test reagent
2. AfinUrimal Result Plate

MATERIALS REQUIRED BUT NOT PROVIDED
1. Test tube
2. Micro pipette
3. Micro pipette tips
4. Sample container

REFERENCES
1. Ifeanyichukwu Okeke, (2022). A Biochemical Assay Provides Better Diagnosis for Malaria Infection. Global Scientific Journals: Volume 10, Issue 9, September 2022, Online: ISSN 2320-9186. Pp: 1839-1853
2. Kemsner P.G., Valinm C., Missinou M. A. et al., (2009).Prognostc value of circulating pigmented cells in African children with malaria," *Journal of Infectious Diseases*, vol.199,no. 1,pp.142-150,2009
3.. Alison Gilchrist, Jim Nobbs, (2017). Encyclopedia of Spectroscopy and Spectrometry (Third Edition).
4. WHO Malaria Parasite Counting, (2016). Malaria Microscopy Standard Operating Procedure – MM-SOP09

SYMBOL LEGENDS

🏭	Afinitest Laboratories	📖i	Consult instruction for use
Σ	Contains sufficient for 50 tests	☀	Keep away from sunlight
IVD	In vitro diagnostic medical device	LOT	Batch code number
↓	Store at 2° to 30 °C	REF	Catalog number
📖i	Consult instructions for use	⊗	Do not use if package is damaged
②	Do not reuse		
LOT			
🏭	MAN EXP		

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FOR SUPPORT AND ENQUIRIES
Call/SMS/WhatsApp:
+234 8161994393