

Can a non-invasive urine-based test become the next-generation diagnostic tool for malaria?

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SUMMARY

This mini review summarises the non-invasive urine-based diagnostic approaches that have been used to diagnose malaria.

Amongst all urine-based diagnosis methods, commercially available Rapid Diagnostic kit/strip is most likely to be suitable for malaria detection in a cost-effective, time-consuming and user-friendly manner. With

further improvement in sensitivity, specificity and accuracy, this technique may become a useful “next-generation gold standard” malaria diagnostic tool in resource-limited regions and in areas where invasive blood tests are restricted.

Keywords: non-invasive, urine, malaria, diagnosis.

OVERVIEW OF CURRENT DIAGNOSTIC METHODS FOR MALARIA

Despite enormous efforts over many years, nearly half of the world’s population is at risk of a malaria infection, with more than one million deaths each year. Timely and accurate diagnostics have great potential to improve the quality of malaria control programs. A worldwide effort is under way to develop new tools for quick as well as effective diagnosis of malaria although microscopy still remains the cornerstone for laboratory diagnosis.

The major constraint of microscopy is the requirement of good technical expertise, especially with low level parasitaemia. Similarly, molecular based diagnostic approach is not routinely used due to high cost, and the need for highly-trained staff though, having high sensitivity and specific-

ity. A good alternative to standard microscopy is now already available in the form of Rapid Diagnostic Kit (RDT) that does not require laboratory, electricity, technical expertise or any special instruments. Currently, the target of RDT is on the detection of Histidine-Rich Protein2 (HRP-2) from *Plasmodium falciparum* and Parasite-Specific Lactate Dehydrogenase (pLDH) or Plasmodium aldolase from all plasmodial species with minimal infrastructure even in highly inaccessible endemic zones. However, like microscopy method, it also faces many difficulties such as inability to quantify and identify low parasitaemia besides, increased risks of needle injuries and disease transmission.

Therefore, the development of an inexpensive, non-invasive rapid method of malaria diagnosis using biological fluids other than blood would provide a more reliable approach.

The non-invasive strategies basically rely on detection of antibodies against plasmodial parasite, parasitic antigens, and their DNA in non-blood samples, *i.e.* urine, saliva and buccal mucosa. Recently, urine based diagnostic approaches were

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under study for evaluating their efficacy in comparison with the benchmarked gold standard reference tools.

■ URINE-BASED DIAGNOSTIC APPROACH: FROM CONVENTIONAL TO MOLECULAR LEVEL

Urine is the liquid excretory product that contains 95% water and 5% water soluble waste compounds such as nitrogenous products (urea, uric acid and creatinine), few hormones, enzymes and mineral salts. Since ancient era, urine has been used as an ideal clinical sample to diagnose infectious diseases. The traditional approach to diagnose most of the disease basically relies on urine colour which is an early indicator of various health issues. Malaria and several other conditions such as glucose 6 phosphate dehydrogenase deficiency and sepsis can change the normal urine colour with a tinge of dark red because of hemoglobin. In case of severe malaria, the red blood cells burst and this can lead to change in urine colour towards coffee brown which in medical terms known as "Black water fever". Earlier reports suggested that varieties of both parasitic antigens and antibodies are possibly released into the urine during malaria infection [1].

The antigenic proteins produced by the merozoite and gametocyte forms of malaria parasite travel from blood to the kidneys, where they pass onto the bladder as part of the urine. Some indirect-immuno-fluorescence results also revealed the presence of plasmodial parasite antigen when antisera raised against urine from *P. falciparum* patients. Similarly, western blot assay also revealed the presence of parasite proteins in the urine of patients with acute *Plasmodium vivax* malaria.

Apart from this, *P. vivax* was also detected in urine sample through a colorimetric system using gold nanoparticles and MSP10 DNA (Merozoite surface protein 10) [2]. Urine from some malaria-infected patients also contain trace amount of plasmodial DNA which was identified by a nested PCR focusing on the mitochondrial cytochrome b gene of the plasmodium species demonstrating some high level of sensitivity compared to other PCR based strategies [3].

The gene copy number may also affect the variation in sensitivity. Finally, development of few RDT kits occurred that can detect the parasite from urine sample of malaria patients besides blood sample. Recently, an UrineMalariaTest™ (UMT) dipstick has been manufactured by Fyodor Biotechnologies, Inc (Baltimore, MD, USA) that detects *P. falciparum* HRP-2 antigenic protein with moderate level of sensitivity when compared with traditional microscopy but showed an improved sensitivity (sensitivity of 83.75% and specificity of 83.48%) compared to other non-specific RDT kits [4].

This kit was initially developed by scientists from Nigeria which was the first non-invasive malaria diagnosis kit clinically evaluated on a large-scale population.

■ PERFORMANCE EVALUATION OF URINE-BASED DIAGNOSTIC TECHNIQUE

Various studies had been conducted, where specificity was found to be 100% for detection of *P. falciparum* and *P. vivax* in urine sample when compared with nested PCR results from blood [5]. Similarly, another study from Gambia showed the detection of small subunit ribosomal RNA gene of *P. falciparum* in urine with higher specificity than that of blood samples [6]. However, the efficacy will further be enhanced by increasing the sensitivity level. Recently, a study was conducted in a malaria endemic State, Odisha of India where the sensitivity level of both RDT urine as well as RDT blood were observed to be almost equivalent [7].

Besides plasmodial antigen, a peptide hormone hepcidine produced by chronic inflammation in the urine of malaria infected individuals also could be a promising biomarker for malaria detection [8].

■ CURRENTLY AVAILABLE URINE-BASED MALARIA DIAGNOSTIC TEST

Among all urine based diagnosis methods, the non-invasive RDT kit/strip (both urine specific and nonspecific) is found to be cost-effective, time-consuming advantageous and easy to use. This method relies on the principle of immu-

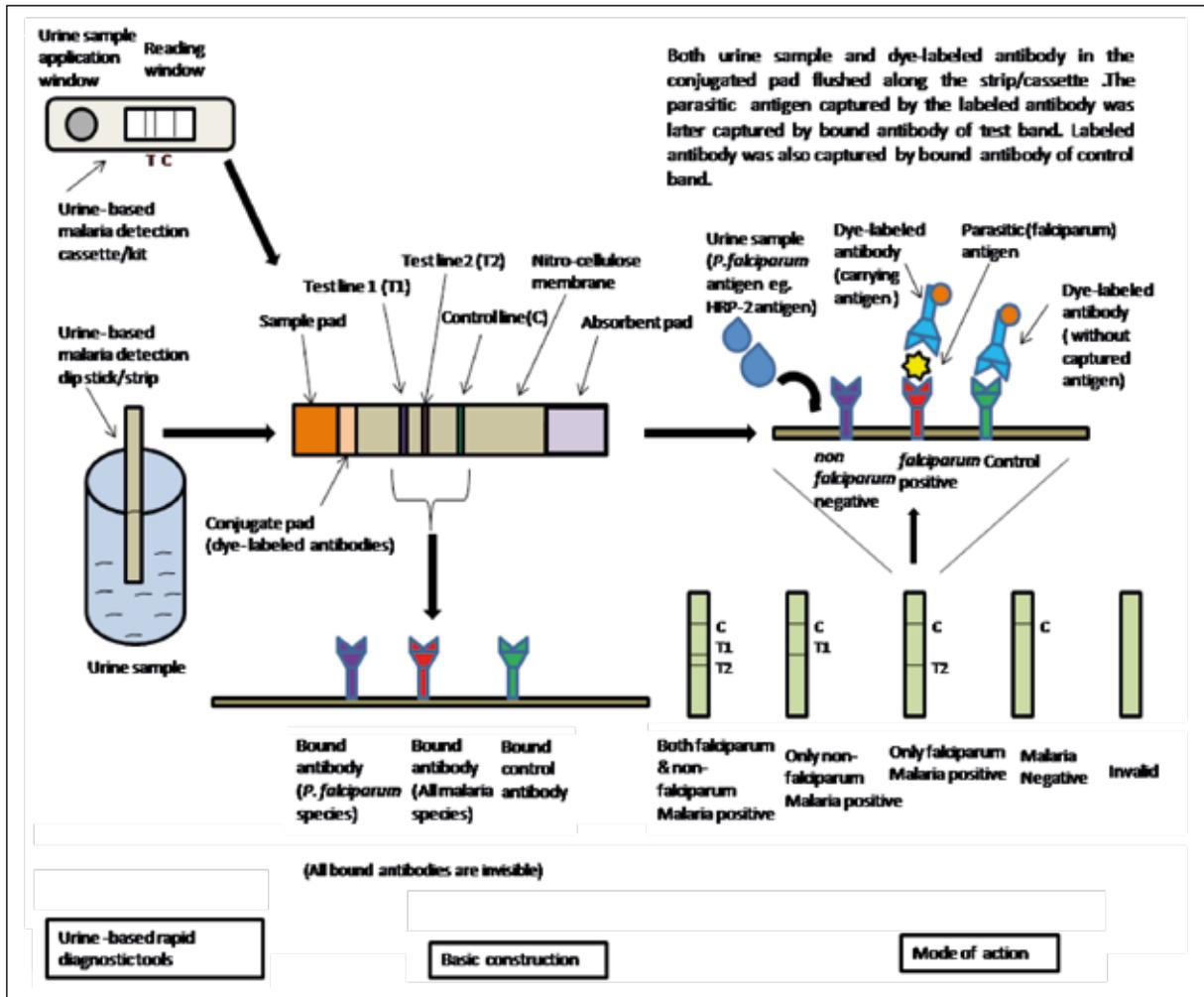


Figure 1 - The present form of urine-based diagnostic tool, its basic construction and mode of operation.

no-chromatography which utilizes recombinant monoclonal antibody to detect parasite antigen protein and fragments in the urine of malaria infected patients (Figure 1).

Amongst rapid diagnostic kits, the UMT dipstick was the first clinically validated urine specific kit that can detect malaria parasite proteins (only Pf specific HRP-2) within 25 minutes. This UMT won the Health Innovation Challenge Awards in Nigeria in the year 2015. Although, findings are still increasing with the use of blood specific malaria RDT kits for body fluids like urine, the overall performance of UMT kit is found to be higher/equivalent to that of blood based malaria RDT [Binax NOW (Pf/Pan)] kit [9].

■ **FUTURE DIRECTION: DEVELOPMENT OF A RAPID, HIGHLY EFFICIENT, COST-EFFECTIVE AND CONVENIENT URINE-BASED APPROACH**

The major milestone of this rapid diagnostic technique is the possibility of self diagnosis at home within a short time period and in a non-invasive manner. Most of the recently available urine-based kits detect only *falciparum* malaria. Hence, the non-*falciparum* malaria might be misdiagnosed as malaria negative. Similarly, the detection level or sensitivity of urine-based RDT kit will higher only at certain parasite density. Therefore, many considerations have to be taken

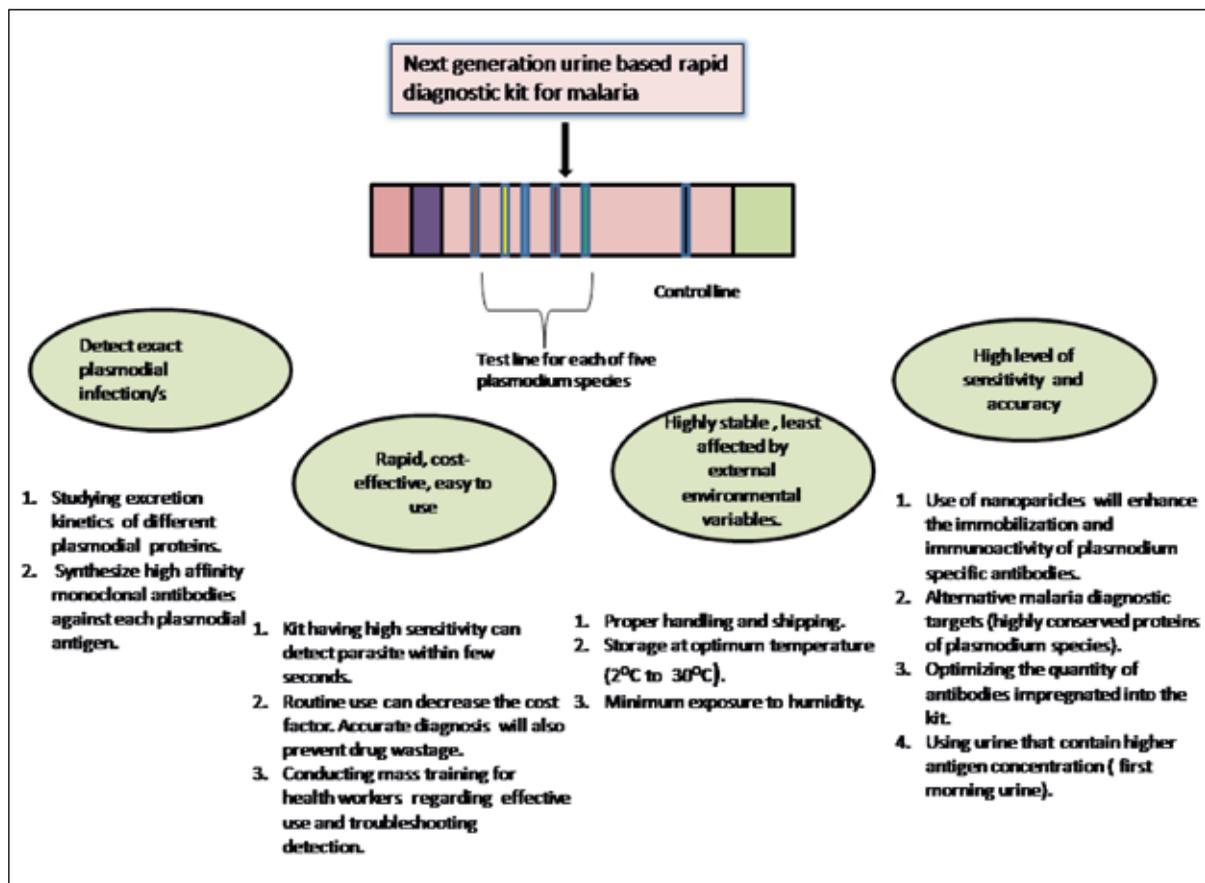


Figure 2 - The next-generation urine-based diagnostic tool and its features.

into account when a new tool/technology is going to be developed (Figure 2). This technology can be further exploited for the development of a highly sensitive, accurate, low cost, convenient as well as rapid and robust tool for malaria diagnosis where invasive blood tests are restricted. Hence, the urine-based approach will not only provide a safety profile in an ethically accepted way but also increase the participation rate in mass malaria screening programs in a couple of years.

■ STATEMENT OF AUTHOR CONTRIBUTIONS

NP designed the main conceptual idea and wrote the review. RKH conceived the study and participated in its design and co-ordination. Both the authors read and approved the final article.

Conflict of interest

The authors have no conflicts of interest.

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