Introduction
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The mosquito is an inimitable vector that can transmit a variety of pathogens ranging from viruses, nematodes to malaria parasites. They are probable to transmit disease to more than 700 million people per year in different part of Africa, Mexico, South and central America and much of Asia; causing millions of death worldwide. In many temperate and developed countries like Europe, Canada, the United States, Australia, Japan, mosquito bites is now mostly an irritating nuisance; but still cause some deaths each year (Mark S. Fradin et al., 1998).

Mosquitoes are perfect examples of one of the many organisms that can host diseases. They cause more human suffering than any other organism, over one million people worldwide die from mosquito-borne diseases every year. Of the known 14,000 infectious microorganisms transmitted by mosquitoes, 600 are shared between animals and humans. Mosquitoes are known to carry many infectious diseases from several different classes of microorganisms, including viruses and parasites. Mosquito-borne illnesses include Malaria, West Nile Virus, Elephantiasis, Dengue Fever, Yellow Fever, Chikungunya etc.

Malaria is an ancient disease probably originating in Africa. The malaria parasite (Plasmodium) is transmitted by female Anopheles mosquitoes. The term malaria is derived from the Italian 'malaria" or "bad air" because it was thought to come on the wind from swamps and rivers. It still remains a major infectious disease causing at least 250 million infections and nearly one million deaths per year (WHO, 2011). An unprecedented survey of the disease suggests that it kills between 125,000 and 277,000 people per year in India alone. Out of 100 known species, four species of Plasmodium can produce the disease in humans i.e. P. falciparum, P. vivax, P. Oval, P. malariae, among them P. falciparum is the most dangerous form of malaria, with the highest rates of complications and mortality. Almost all malarial deaths are caused by P. falciparum. Malaria is much more prevalent in sub-Saharan Africa than in other part of the world; in most African countries, more than 75% of reported cases were due to P. falciparum. The malaria parasite P. falciparum is one of the major causes of child mortality worldwide, which annually kills 11 million children in Africa alone and estimated to cause about half a billion episodes of disease each year (Snow et al., 2005), and there are hundreds of millions of cases due to other parasite species P. vivax, P. malariae, and P. ovale. In regions of high malaria transmission, every member of the community might be chronically infected (Trape et al., 1994). The ability to accurately and rapidly diagnose malaria infection is essential to the success of elimination and control of the disease. Microscopic examination of blood smears
has still the traditional and gold standard method for detecting malaria parasites. The accuracy of microscopy largely relies on the experience and training of the microscopist and the quality of smears. The quality of the microscopy varies significantly and is often unreliable. Importantly, it is hard to maintain good quality microscopy especially in remote areas where malaria occurs commonly.

An accurate diagnosis is becoming more and more important, in view of the increasing resistance of *P. falciparum* and the high price of alternatives to chloroquine. Rational therapy of malaria is essential to avoid non-target effects, to delay the advent of resistance, and to save cost on alternative drugs. Confirmatory diagnosis, before treatment initiation, recently regained attention. This is because of an increase in drug resistance and thus the requirement of more expensive drugs, which are unaffordable by poor countries. Microscopy and rapid diagnostic tests (RDTs), the two malaria diagnostics that is likely to have the largest impact on malaria control today. Recently, several new rapid diagnostic tests have been evaluated in which one of the most prominent alternatives to microscopy has been a series of tests based on the detection of the histidine rich proteins (HRPs) (Sullivan DJJ et al., 1996; Taylor DW et al., 1993). This can be performed with rapid Immunochromatographic formats currently available such as, the Para Sight F test (Becton Dickinson, Meylan, France) or the MalaquickTM test (ICT, Sydney, New South Wales, Australia). In practice, these tests are reported to perform well (Garcia M et al., 1994; Beadle C et al., 1994; Rabodonirina Ugen C et al., 1995). However, the HRP-2 antigen is present only in *P. falciparum* infections and thus the test cannot be used for the detection of *P. vivax* or other human malarias (*P. ovale* and *P. malariae*). The antigenic activity of HRP-2 has also been found to persist in the bloodstream long after a malarial episode has been resolved by antimalarial therapy.

Malaria antigen detection tests are a group of commercially available tests that allow the rapid diagnosis of malaria by people who are not otherwise skilled in traditional laboratory techniques for diagnosing malaria or in situations where such equipment is not available. The first malaria antigen suitable as a target for Rapid Diagnostic Tests (RDTs) was a soluble glycolytic enzyme glutamate dehydrogenase (Ling IT et al., 1986; Rodriguez Acosta A et al., 1998; Ning Li Y et al., 2005). A major drawback in the use of all dipstick methods is that the result is essentially qualitative. Antigen detecting assays are popular now a day as they are capable to detect lower parasite count (quantitative measurement) and produce quick results without any extensive training or any sophisticated equipment. The quantitative assessment of parasitemia is important in many endemic areas of Africa and Asia because a large percentage of the population may test positive in any qualitative assay and produce a false diagnosis. A number of antigens from the asexual blood stage of malaria parasites are considered
as remarkable candidates for development of diagnostic reagents. Out of these, two antigens are reported to be promising in laboratory as well as in field study. These unique antigens are *P. falciparum* histidine rich protein-2 (HRP-2) and Lactate dehydrogenase.

HRP-2 is a Histidine and alanine rich, *P. falciparum*-specific water-soluble protein, localized in the parasite cytoplasm and on the surface membrane of infected erythrocytes. It is present on the protrusions, known as knobs, thought to account for sequestration of the trophozoite and schizonts in post capillary venules. There is increasing concentration of HRP-2 as the parasite advances from ring stage to trophozoite and it readily diffuses into the plasma (*Iqbal J et al., 2000; Beadle C et al., 1994*). HRP-2 from *P. falciparum* has been implicated in the biocrystallization of hemozoin, an inert, crystalline form of ferriprotoporphyrin IX (Fe(3+)-PPIX) produced by the parasite. A substantial amount of the HRP-2 is secreted by the parasite into the host bloodstream and the antigen can be detected in erythrocytes, serum, plasma, cerebro-spinal fluid and even urine as a secreted water-soluble protein (*Rock EP et al., 1987*). These antigens persist in the circulating blood after the parasitemia has cleared or has been greatly reduced. It generally takes around two weeks after successful treatment for HRP-2 based tests to turn negative, but may take as long as one month, which compromises their value in the detection of active infection (*Humar A et al., 1997*). False positive dipstick results were reported in patients with rheumatoid-factor-positive rheumatoid arthritis (*Iqbal J et al., 2000*). Since HRP-2 is expressed only by *P. falciparum*, these tests will give negative results with samples containing only *P. vivax*, *P. ovale*, or *P. malariae*; so HRP-2 based diagnostic tests are highly specific for *P. falciparum*, a most dangerous form of malaria. The other advantage of this HRP-2 antigen based diagnostic assay is it does not show any cross reactivity with other species of malaria parasite.

Another antigen target to detect sexual and asexual stage malaria parasites is *Plasmodium* lactose dehydrogenase (pLDH), which is the terminal enzyme in the malaria parasite’s glycolytic pathway. The lactate dehydrogenase (LDH) of malaria parasites is immunologically distinct from the host enzyme thus suitable for accurate diagnosis of malaria in field conditions. The pLDH isoform can be distinguished from the human isoforms on the basis of unique epitopes within the pLDH protein, as well as on its enzymatic characteristics (*Vander Jagt et al., 1993*). pLDH is correlated with the number of parasitemia present in the plasma of malaria infected patients (*Markler and Hinrichs DJ, 1993*). It is expressed at high levels in asexual stage or blood-stage parasite and present in all four malaria parasites (*Vander Jagt DL et al., 1990; Vander Jagt DL et al., 1981; Sherman IW, 1979*). It was found that all four
human malarial parasites produce a unique pLDH activity i.e it is described that an enzymatic assay that can specifically measure pLDH in the presence of human LDH. This test is based upon the fact that pLDH can use the 3-acetyl pyridine analog of NAD (APAD) while human LDH isozymes do not readily use this analog (Makler MT et al., 1993). This activity follows the level of parasitemia in in vitro cultures (Vander Jagt et al., 1993; Makler MT et al., 1993), indicating that pLDH may be a good marker for following active malarial infections. Thus, pLDH activity can be readily measured in dilute whole blood lysates using the MalStat reagent, which contains APAD (Makler MT et al., 1993). However, measurement of pLDH activity is complicated by the presence of hemoglobin, which interferes with colorimetric detection of pLDH activity (Knobloch J et al., 1995; Jelinek T et al., 1996). Consequently, the use of the MalStat assay is restricted to use as a simple method for detecting growth of parasites in invitro drug susceptibility testing.

The L-lactate dehydrogenase of *P. falciparum* (P/LDH) is essential for the anaerobic lifestyle of the *Plasmodium*, (Royer et al., 1986). The enzyme has three strikingly different kinetic properties compared with most LDHs studied to date. (i) Unlike the human version, the malarial enzyme is not inhibited at high pyruvate concentrations (Vander Jagt et al., 1981). (ii) Unlike the human protein, the malarial enzyme is very active with a synthetic coenzyme, 3-acetoylpyridine adenine dinucleotide (APAD), at 0.5 M lactate (this is the basis of a test for parasitemia in human blood (Makler and Hinrichs, 1993). (iii) The malarial enzyme is much more sensitive to inhibition by gossylic nitrile diacetate than the human equivalent. The N-terminal amino acid sequence of pLDH has revealed that LDH is structurally different from human and other vertebrate and bacterial LDH. In particular, an insertion of five amino acid residues in the active site loop creates an enlarged volume in the substrate binding site, and characteristic changes in the residues lining the NADH cofactor binding pocket result in displacement of the co-factor relative to its observed position in mammalian and all other LDH structures. These results imply the special features previously described for P/LDH may be shared across the Plasmodium genus, supporting the universal application of therapeutics targeting this enzyme (Winter VJ et al., 2003). Although the Michaelis-Menten constant (Km) are similar, the turnover number (Kcat) of the pLDH is much more than human enzyme (LDH) in the presence of same co-factor i.e. 3-acetoylpyridine adenine dinucleotide (APAD). This is due confirmatory changes occurs in the enzyme, which is involved in the rate limiting step for the oxidation reduction (Dunn CR et al., 1996; Gomez et al., 1997). *P. falciparum* lactate dehydrogenase (pLDH) is a 33 kDa oxidoreductase (Bzik DJ et al., 1993). It is the last enzyme of the glycolytic pathway, essential for ATP generation and one of the most abundant enzymes expressed by *P. falciparum* (Vander Jagt DL
et al., 1981). Plasmodium lactate dehydrogenase does not persist in the blood but clears about the same time as the parasites following successful treatment (Iqbal J et al., 2004). The lack of antigen persistence after treatment makes the pLDH test useful in predicting treatment failure. In this respect, pLDH is similar to pGluDH. LDH from P. vivax, P. malariae and P. ovale exhibit 90-92% identity to pLDH from P. falciparum (Brown WM et al., 2005).

The detection of parasite antigen has been considered as a better alternative for the quantitative as well as qualitative estimation of malaria in human. HRP-2 and pLDH is found to be a novel marker for development of ELISA based diagnostic assay. So the aim of the present study is to develop an Enzyme Linked Immunosorbert assay for the detection of HRP-2 and pLDH antigen in malaria infected patients.

Chikungunya (CHIKV) virus is another disease, transmitted to humans through mosquito bites. After the epidemics of dengue and SARS, now chikungunya became the biggest scare in the world currently and grabbed the attention of the people worldwide. The name chikungunya is derived from the Swahili word meaning "that which bends up". The disease, caused by the CHIKV has already involved in many outbreaks in Africa and Asia ever since its discovery in 1952 in Tanzania (Edelman et al., 2000; Powers and Logue, 2007). Then an outbreak was seen in Portland in Malaysia in 1999 affecting 27 people. It is endemic in parts of Africa (Transvaal, Uganda, Congo, Nigeria, Ghana, Zimbabwe, Senegal, Burkina Faso, and Cameroon), Southeast Asia (Philippines, Malaysia, and Cambodia) and the Indian subcontinent (Pakistan and southern India). There is no direct person to person spread. The mosquito Aedes aegypti is the main vector for the transmission of disease. In the outbreak occurring in Italy in 2007, Aedes albopictus, which is commonly found in Hong Kong, was also identified to be able to transmit the disease. The mosquito likes to bite people during the day time, especially two hours after sunrise and a few hours before sunset. In India CHIKV was first isolated in Calcutta in 1963, after that episode, there have been several reports of CHIKV infection in different parts of India. More than 1,80,000 cases have been reported to occur in India since December 2005.

CHIKV produces a dengue-like illness in humans, characterized by fever, rash, and severe arthralgia; the illness is usually self-limiting and lasts for 3-10 days, although the joint pain may last for weeks to months. The most significant characteristic of CHIK is the prolonged arthralgia syndrome that primarily affects the peripheral small joints associated with excruciating pain (Powers and Logue, 2007). Serological and antigenic characterization of the etiological agent
collected during the epidemic concluded that the alpha virus found to be closely related to Mayaro and Semliki Forest virus (Powers and Logue, 2007).

CHIKV is an enveloped, single-stranded positive polarity RNA virus with a genomic size of approximately 11.8 kb (Sudeep A B and Parashar D, 2008). A subgenomic positive-strand RNA referred to as 26S RNA, identical to the 3' of the genomic RNA, is transcribed from a negative-stranded RNA Intermediate. This RNA serves as the mRNA for the synthesis of the viral structural proteins. According to the genomic organization of other alpha viruses, the genome of CHIK is considered to be: 5' cap-nsP1-nsP2-nsP3-nsP4-(junction region)-C-E3-E2-6K-E1-poly(A)3'. E1 and E2 have now been considered as two major glycoprotein's responsible for infection. E1 glycoprotein is responsible for cell membrane fusion, while E2 is primarily involved in receptor binding and cell entry as well as eliciting neutralizing antibodies.

No vaccine or specific antiviral treatment for chikungunya fever is available. Treatment is symptomatic which includes, rest, fluids, and ibuprofen, naproxen, acetaminophen, or paracetamol may relieve symptoms of fever and aching. CHIKV produces disease about 48 hours after the mosquito bite. Patients have high viraemia during the first 2 days of illness. Viraemia declines around 3 or 4 days, usually disappearing by day 5 (Carey DE et al., 1969; Shah KV et al., 1964). Haemagglutination inhibition (HI) and neutralizing antibodies can usually be detected after day 5 with fading viraemia (Carey DE et al., 1969). Clinical laboratory findings are not remarkable, few patients may present with leucopenia with relative lymphocytosis; however, most patients will have a normal blood count. The platelet count may be moderately less. Erythrocyte sedimentation rate is significantly elevated and C-reactive protein is positive in acute cases.

Confirmatory diagnosis of Chikungunya before treatment initiation is essential to avoid non target effect of drugs as it shows similar clinical symptoms as dengue and other alpha viruses. Despite the above disease burden the diagnosis is still based on leucopenia, anemia and elevation of serum aminotransferase enzymes. However, none of the above laboratory features are specific for the diagnosis. Detection of chikungunya can be done by molecular methods such as RT-PCR (Smith DR et al., 2009) and virus culture (Couderc T et al., 2008). RT-PCR real time loop mediated isothermal PCR (Parida MM et al., 2007), antigen capture ELISA (Shukla J et al., 2009). These methods are highly sensitive but can be used only within the first week of disease onset. Cell culture also allows potential isolation of a wide range of viruses. RT-PCR can detect nucleic acid from CHIKV but it is useful for blood samples obtained beyond three days. However, once the patients start producing antibodies,
the probability of a positive culture or PCR decreases. As well due to lack of proof reading, it is sometime difficult to design the primers for detection of virus RNA using RT-PCR. Additionally, the high cost of RT-PCR analysis is also another problem associated with small laboratories. Classical serological assays like IgM detection assay (Gerardin et al., 2008), IgG based immunolateral flow assay (Rampal et al., 2007), Immunoflorescence (Cho B et al., 2008), Complement binding and haemagglutination inhibition method (Clarke DH et al., 1958) has also been reported, but they lack sufficient specificity and need trained person.

We intend to improve the specificity of the existing serological method by detecting antibodies to immunodominant peptides instead of using the whole protein. However, using peptide based ELISA in the diagnosis is very scanty in the literature. The usefulness of peptides for the diagnosis of viral, bacterial, parasitic and autoimmune diseases has been demonstrated by many workers (Alcaro MC et al., 2003; Gomara MJ et al., 2007; Noya O et al., 2003; Shweta H Morey et al., 2010; Deepak Tomar et al., 2006). Synthetic peptides offer the advantage of enhanced specificity and eliminate nonspecific reactions by avoiding the cross reactive sequences.

Therefore, there is a need of cheaper as well as a sensitive diagnostic tool with less time to detect antibodies in the sera of clinical samples. Thus an urgent need to develop diagnostic reagents to diagnose Chikungunya without cross reactivity with other related alpha viruses. The commercial kits are based on detecting IgM antibodies using recombinant envelope proteins. IgM antibodies appear between 4-5 days after onset of fever. In convalescent sera, IgG starts appearing from 10th days of clinical symptoms and can persist up to months (Gilles Pialoux et al., 2007). In infected patients viraemia is present in serum that lasts up to 6 days whereas symptoms appear 4-6 days. There are reports where IgM antibodies can be detected up to month’s days after Chikungunya fever (Taubitz et al., 2007). Serological detection of IgM and IgG is most useful in the retrospective diagnosis of Chikungunya infection. In the present study we aim to develop peptide based detection of anti-CHIKV antibodies with improved sensitivity and specificity from the existing serological assays.

Hence, the objective of this study is to identify peptide specific antibodies of E1, E2 and E3 protein to diagnose the CHIK infection using in-house reagents.