

9. Diagnostic Tools

9.01 A novel capillary-like microchannel for rapid diagnosis and microrheology of human malaria parasite *Plasmodium falciparum* erythrocytes

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We present a new diagnostic system which accomplishes both the malaria parasite-engineered structural and the parasite-viscoelastic changes based on a novel micro-channel according to the capillary blockage of the infected RBCs. The present study has successfully detected the deformation and the plane motion of the infected cells by a micro-channel approached the size of the single cell. However, with the merits of bio-fluidic technique, the well-controlled pressure and viscoelastic detection are embedded into our fluidic system, thus resulting in potentially accuracy diagnosis. The 'step-size' PDMS (polydimethylsiloxane) channels ranging from 2 to 8 μm in width was fabricated, and the height of the channels was chosen as 6 μm uniformly that provides the freedom for RBCs' 2.5D-rotation but fixed in a same z-axis focussing plane. Then, the PDMS channel was sealed with a cover-slip and connected to the reservoir which provides fine pressure difference by varying the height of the water level in upstream and downstream. As the result of our experiment, we observed the deformed shape and blockage of infected RBCs in different stage. We also observed the squeezing of healthy cells through the aggregate infected cells. In the viscoelastic properties of the infected RBCs, we tracked the merozoites according to the particle-tracking microrheology method of Mason *et al.*, which provides us the frequency-dependent spectrum of viscoelastic shear moduli of the cell. Finally, we found that under the restriction of the channel wall, the MSD (mean square displacement) and the elastic modulus of the infected cells increased significantly. In summary, the viscoelastic property was proven related to the change of mechanical property during the deformation of the infected cells. We also demonstrated our new bio-fluidic platform exhibits bio-compatible, MEMS low cost, and constructing a testing environment like the real human capillary.

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9.02 Screening of blood donors to prevent transfusion transmitted malaria in India

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Transfusion transmitted malaria poses significant problem regions of world where malaria is endemic. This may result in significant morbidity and mortality in transfusion recipients. In India though it is mandatory by drug and Cosmetic act to screen donated blood for malaria, there are no definite guidelines on the choice of the test. Donors who are implicated as the source of transfusion transmitted malaria cases typically have very low level of parasitemia undetectable even on several thick films. Moreover, traditional blood film microscopy involving large number of blood donor samples is labour intensive and requires high technical skill. Malaria antibody screening is not indicative of active infection and result in unnecessary high discarding of collected blood units as the antibody may persist up to several years after infection. PCR and antigen detection tests have limited availability. Hence, most of the donated blood across the country is not screened for malaria and experts recommend malaria immunoprophylaxis to all blood recipients which is not feasible practically. This practice may also pose significant problem in immunosuppressed patients, neonates and children. Reports on transfusion-transmitted malaria from India are not available as in the absence of awareness, the cases may be attributed to the mosquito-acquired malaria. High prevalence of malaria in multi transfused thalassemia patients points towards transfusion as a risk factor. We report on prevalence of *Plasmodium falciparum* and *P. vivax* antigen screening by rapid test in our donor population and multi transfused thalassemia patients. The findings call for definite guidelines on blood screening to prevent transfusion-transmitted malaria.

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9.03 Prevalence of *Plasmodium falciparum* in peripheral smear of pyrexia patients at OPD of Agartala Government Medical college G B Pant Hospital during 2003-2005 March

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Malaria is a major public health problem. For diagnosis of malarial parasites, direct microscopy of peripheral smear is widely used. To study the epidemiological distribution of *P. falciparum* with its positive rate and also to compare with Hb_{gm}% label to use as parameter. Based on direct microscopy of peripheral blood smear. 7,490 patients having history of fever were registered in outpatient department of Agartala Government Medical college hospital (G. B. Pant hospital) during March 2003 - 2005. Blood was collected aseptically in EDTA vial, smear was prepared of all 7,490 registered cases and leish man stain were done Hemoglobin estimations were done by acid hematin method for all 36 positive cases in the department of clinical microbiology. Out of 7,490 smears 36 were positive. Among the 36 positive patients, male are more than female having 66.66% and 33.33% respectively. Smear shows *Plasmodium falciparum* positive rate 86.11%, others *vivax* 8.33% and gametocyte 5.55%. Hemoglobin pattern is markedly decreased below 8% is 66.66% and below 10% and above 8% were seen 36.11% cases only. Mostly were treated at home. Detailed drug history is not known.

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9.04 Detection of HRP-II antigen from *Plasmodium falciparum* by latex agglutination test

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Malaria is one of the most prominent vector borne diseases. Malaria is responsible for 500 million cases of clinical diseases globally and presents a public health problem for 2.4 million people representing more than 40% of the world's population in over 90 countries. Early diagnosis and treatment of parasitemia is vital for the control of malarial infection. For the detection of malarial parasite techniques like microscopic, non-microscopic and molecular tools are generally used in laboratory and field. Microscopic peripheral blood smears and quantitative Buffy coat and molecular methods like PCR and labelled immuno assay are not used in field. Microscopic examination of blood smear is the golden standard method for malaria diagnosis. Other rapid non-microscopical assays for detection of malarial infection available based on antigen released from parasitized red blood cells. *Plasmodium falciparum* Histidine rich protein-II (HRP-II) is a soluble antigen produced only by *P.falciparum*, but other species including *P.falciparum* can synthesise lactate dehydrogenase (pLDH) which is different from human LDH. HRP and LDH based kits are being used in field for the detection of malarial infection. HRP-II gene of *P. falciparum* was cloned (pQE-30 UA), expressed and protein was purified by recombinant technology in *E. coli*. The above protein was subsequently used to make diagnostic reagent where other immunological techniques were involved. Antibody (Titre 1:25600 by dot-ELISA) coated nano (latex 0.93 μ) particles were used to detect the HRP-II antigen in the field collected blood samples of suspected malaria cases. The results showed that as low as 125mg of antibody coated latex particles was in a position to detect parasite antigen from the field-collected samples and well compared with microscopic and other commercial available kits. The positive samples showed a clear agglutination reaction within 2-3 minutes compared to control samples. There was no agglutinin particles seen visibly. The field-based kit was developed and found to be simple, rapid and cost effective.

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9.05 Pattern of vector born parasitic infection in Tripura - diagnosed by QBC assay

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Tiny state of Tripura is a known endemic zone for malarial infection with high mortality & morbidity during peak transmission season. Predominant vector in rural area is *Anopheles dirus* & *minimus*, *Culex species* also available. In order to improve detection of hemoparasites, QBC Assay was introduced in Tripura in private laboratory since July, 2000. During these 5 year period, total no of 6449 blood samples were examined by QBC Assay. Samples were received from patients with pyrexia of acute onset as well as PUO patients. QBC capillaries processed as per standard protocol. Giemsa stain done in thin smear for confirmation of species. Out of 6449 samples, 1349 (21%) were positive for malaria. Among them, 1072 (79%) *P.falciparum*, 197 (15%) *P.vivax*, 80 (6%) mixed infection. Surprisingly, in 4 patients *Microfilaria* was detected where clinical suspicion was for malaria though not in indigenous people. This is first time in Tripura *Microfilaria* could be detected from day time samples because of using sensitive diagnostic tool. Incidence of malaria in Tripura is quite high *P.falciparum* is the predominant species. Appropriate intervention is needed.

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9.06 Use of dipstick /quantitative buffy coat technique (QBC) for diagnosing malaria in public & private laboratories

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This study was done in Surat city in the year 2000. Aim of this study was to see, what other methods are being employed in public & private laboratories for diagnosing Malaria, apart from conventional microscopy. Microscopists of 88 public & private laboratories, who were involved in Malaria microscopy, were interviewed with a pre-designed questionnaire & the key question asked was whether, they were using any other new methods like Dipstick/QBC etc. for diagnosing Malaria. It was found that only 16 laboratories out of 88 were relying upon dipstick & QBC.

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9.07 Description of an on-going collaborative study for the characterisation of an International Standard for *Plasmodium falciparum* Nucleic Acid Amplification Assays

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This abstract describes an on-going collaborative study for the characterization of a candidate International Standard for *Plasmodium falciparum* nucleic acid amplification assays. This study is also to determine the parasite content of the candidate standard. Due to the number of different Nucleic Amplification Technique (NAT) assays available, a degree of standardization is needed to ensure assays are recording results equally and accurately. The aim of this study is to produce a Standard that all assays can be tested against. At present there are 14 participants in this study with furthers participants expected to join. Participants are asked to assay 4 separate samples ranging from a freeze-dried preparation, an *in vitro* culture sample, and 2 clinically infected blood samples. Results from this study will be analyzed statistically and a unitage will be assigned to the proposed standard. The results of the study are then submitted to the WHO Expert Committee on Biological standardization (ECBS), who then decide if the preparation is suitable to become an International Standard.

Allah auquels je suis allé.
Un mot d'abord sur le manuscrit dont j'ai
été conduit à rechercher en détail dans le
cours des maladies affectées d'impureté.
Et mon séjour en Algérie, en 1879, j'ai
été conduit pour pour but l'étude de
l'anatomie pathologique des foyers purulents et
personne je pense en ce moment grand

Manuscript by Laveran (fragment), for a communication to the
Medical Society of Hospitals on April 28, 1882