Project title: *Multiplexed Point-of-Care test for acute febrile illness (mPOCT)*

Project summary:*

Acute fever or acute febrile illness (a rapid onset of fever and symptoms such as headache, chills or muscle and joint pains) is common in the tropics and sub-tropics and can be caused by very diverse pathogens[1-3]. Differential diagnosis of these etiologies based on clinical criteria alone is not possible as clinical signs and symptoms of most of these infections are very similar and the correct diagnosis is only possible by using pathogen specific diagnostic tests. For patient treatment and management, differential diagnosis of causative agent is required [1-3]. In low income countries, many preventable deaths occur because of delayed or lack of correct diagnosis. In last few years, extensive efforts to control malaria are resulting in positive outcome. In fact, non-malarial febrile illnesses (NMFI) cause more deaths than malaria even in malaria-endemic countries and in the absence of accurate or available diagnostics for NMFI, many non-malarial fevers are treated as malaria which is contributing in the generation of artemisinin resistance [1, 2, 4, 5]. Based on these facts, availability of multiplex test which can quickly identify a pathogen from a group of pathogens that cause the similar symptoms is of paramount importance not just from medical standpoint but will also have much greater public health relevance[1].

There are many state of the art diagnostic platforms and techniques available which can be used for multiple target screening in a specimen e.g. advanced multiplex nucleic acid tests, array based immunoassays and bead/flow based assays [6-8]. Unfortunately, these platforms are not suitable for most of the developing countries as these tests tend to involve complex equipment, are expensive and not proven to be robust in field situation where constant power supply is problem and regular maintenance is a challenge. In the resource-limited settings, the impact of diagnostic tests that can be provided at immediate point-of-care is potentially even greater, because the alternative to a POC test (POCT) may be no diagnostic support at all [8]. Based on these facts, in this proposal, we have decided to use simple field deployable lateral flow formats, which with some innovation, can be used for the generation of multiplex test for at least 5-6 major high-burden pathogens responsible for AFI in tropical and subtropical regions of the world especially SEARO region. Based on literature search, infectious diseases which cause major burden of AFI and also amenable to multiplexing include Malaria, Dengue, Typhoid/Paratyphoid, Chikungunya, Leptospirosis and Scrub Typhus [1-3, 5, 9]. These are the diseases that are proposed to be targeted by multiplex POCT.

Despite the strong need, no multiplex POCT is available in market which can be used in resource limited settings for the detection of multiple etiologies of AFI. Although, individual (singleplex) POCTs for the chosen infections (Dengue, Malaria, Typhoid/Paratyphoid, Chikungunya, Scrub Typhus, Leptospirosis) are commercially available but most of these tests are of poor quality. Only the POCTs for malaria (because of FIND/WHO extensive evaluation program), and to some extent Dengue NS1 Ag, fulfill WHO ASSURED criteria [1, 5, 10]. The POCTs for infectious diseases developed in developed countries are often imported by developing countries but these tests are generally very expensive and also do not perform to the mark in the developing countries. Major reasons for poor performance of available tests are: 1) Not sufficient financial incentive to develop high quality rigorously evaluated tests for developing countries; 2) Use of poor quality antigen/antibodies; 3) lack of knowledge about specific target(s) of particular pathogen which causes problem of cross-reactivity; 4) Lack of evaluation using local clinical specimen (inappropriate cutoffs) [8, 10, 11]. Because of the problems in available singleplex tests, we also propose to generate high quality diagnostic intermediates/reagents for each pathogen. Here, we also propose to generate, affordable handheld mobile phone based test reader which will improve both the sensitivity and specificity of the test as the reader will remove the subjectivity involved in reading the test line or dot.
**Strategy:** The strategy will involve parallel/simultaneous detection of IgM antibodies against particular pathogen and pathogen specific antigen in whole blood or serum. For *Plasmodium falciparum* and *P. vivax*, antigen will be detected. For Dengue, both antigen and IgM will be detected. For *S. Typhi/ Paratyphi A*, *Leptospira spp.*, *Orientia tsutsugamushi* and Chikungunya virus only IgM antibodies will be detected. We will utilize fused strip approach for multiplexing as this approach allows incorporation or removal of any target from the panel, without affecting the performance for other targets. This is very important as the prevalence of different pathogens varies between regions. Another advantage of fused strip approach is that it’s an open system and can be manufactured by many diagnostic companies present in developing countries without any IP issues related to the platform. In this project, we will also develop a mobile based assay reader which will improve both the sensitivity and specificity of the test as the reader will remove the subjectivity involved in interpreting the results. The reader will also remove dependence on colloidal gold tracer which is not very sensitive. The proposed reader will also be capable of transmitting data to central server which will help in disease surveillance and will have greater public health significance. The high quality multi-country sera panel for major febrile illnesses will also be generated in this project. Evaluation of assay using clinical samples from developing world/SEARO region is prerequisite as regional background must be determined to tune the cut-off value [10]. The whole project will be guided by the WHO ASSURED criteria. THSTI, India will play role of coordinator (nodal point) for this project.

*As taken from original proposal template, question 5.*

**References**