Validation of ELISA MAC IgM in study sample

Enrolment of study participants with fever of unknown cause using inclusion and exclusion criteria

MAC ELISA IgM assay developed using archived sera controls

MAC ELISA IgM optical density (OD) cut-off calculated by ROC after performing ELISA MAC IgM on archived sera controls known to be Leptospira positive (18 sera) or negative (50 sera) by MAT

MAC ELISA IgM cross-reactivity with other pathogens determined by performing MAC ELISA IgM on 34 archived sera from patients with other infections. When MAC-ELISA IgM positive, MAT performed to exclude Leptospira co-infection

MAC ELISA IgM and MAT performed on acute and convalescent sera. Positive acute or convalescent ELISA MAC IgM defined as OD reading above the determined cut-off at an analyte titre of $\geq 1:400$ on serial dilutions of sera. Paired sera positivity defined as four-fold rise in titre or a positive acute or convalescent sera by above criteria

MAC ELISA IgM sensitivity, specificity, PPV and NPV determined for acute, convalescent and paired sera with MAT as gold standard

After satisfactory performance with controls, MAC ELISA IgM then validated on study sample of patients with unknown febrile illness