Introduction: There is a need for improved diagnostics since standard tests for leptospirosis are not widely accessible and have low sensitivity during early-phase illness. Lig proteins were shown to be high-performing serodiagnostic markers for leptospirosis in immunoblot and ELISA studies. In this study, we evaluated the feasibility of developing a rapid Lig protein-based assay.

Methods: We cloned and purified four recombinant fragments of *Leptospira interrogans* serovar Copenhageni LigA and LigB, which were applied to a dual-path (DPP) platform, a two-dimensional lateral flow format which yields results in 15 minutes. We evaluated the prototype using sera from hospitalized cases of leptospirosis, blood bank donors and patients with dengue, hepatitis virus A and hantaviral infections from Brazil. Results: A DPP assay, formulated with a combination of the C-terminal repeat domain portions of LigA and LigB, showed higher performance than assays based on single fragments or other combinations. The sensitivity of the DPP prototype was 81% (71/88; 95% CI, 71-88%) and 95% (21/22; 95% CI, 75-100%) among the acute-phase leptospirosis ≤ 7 days and > 7 days. The specificity of the prototype was 100% (39/39; 95% CI, 89-100%) and 85% (51/60; 95% CI, 73-93%) among blood bank donors and control patients from endemic area, respectively. Conclusions: A Lig protein-based DPP prototype assay achieved high sensitivity and specificity for diagnosing leptospirosis. These findings need to be confirmed in patient populations with mild disease manifestations and other epidemiological settings. Yet, the results for the assay suggest that development of an effective rapid diagnostic test for leptospirosis may be feasible, which in turn may enable efforts to improve early case detection, implement timely treatment with antimicrobial agents and decentralize laboratory case confirmation during surveillance.