Rapid and accurate visceral leishmaniasis (VL) diagnosis is needed to initiate prompt treatment to reduce morbidity and mortality. Here, we evaluated the performance of loop-mediated isothermal amplification (LAMP) assay for the diagnosis of VL from blood in an endemic area in Ethiopia. LAMP was positive in 117/122 confirmed VL cases and negative in 149/152 controls, resulting in a sensitivity of 95.9% (95% CI: 90.69–98.66) and a specificity of 98.0% (95% CI: 94.34–99.59), respectively. The sensitivity of the LAMP assay was 95.0% (95% CI: 88.61–98.34) in HIV-negatives and 100% (95% CI: 85.18–100.0) in HIV-positives. Compared with microscopy, LAMP detected 82/87 (94.3%, 95% CI: 87.10–98.11) of the microscopy1 cases and was negative in 11/27 (40.7%, 95% CI: 22.39–61.20) of the microscopy2 cases. Compared with the rK39 serology, LAMP detected 113/120 (94.2%, 95% CI: 88.35–97.62) of the rK391 cases and was negative in 149/154 (96.8%, 95% CI: 92.59–98.94) of the rK392 cases. However, when compared with microscopy only, rK39 detected 83/87 (95.4%, 95% CI: 88.64–98.73) of the microscopy1 cases and negative in only 12/27 (44.4%, 95% CI: 25.48–64.67) of the microscopy– cases. There was an excellent agreement between rK39 and LAMP (Kappa 5 0.91, 95% CI: 0.86–0.96). Furthermore, an algorithm using rK39 followed by LAMP would yield a sensitivity of 99.2% (95%CI: 95.52–99.89) and a specificity of 98.0% (95% CI: 94.34–99.59). The findings demonstrate that LAMP assay is an accurate and rapid molecular assay for VL diagnosis, including in HIV-1 co-infected patients, in an endemic setting.