Tuberculosis (TB) cellular immune responses were examined in the breast milk of human immunodeficiency virus infected mothers using the T-SPOT®. TB interferon-gamma release assay (IGRA). Positive TB interferon-gamma (IFN-γ) responses were detected in 6 of 8 (75%) valid breast milk assays. Among 7 mothers with paired breast milk and blood assays, TB IFN-γ responses were higher in breast milk than in blood (P = 0.02). The magnitude of TB IFN-γ responses in maternal breast milk and blood were correlated. Elucidating the influence of TB immune responses in breast milk on infant TB susceptibility and immunity may inform future maternal TB vaccine strategies.