Herpes simplex encephalitis is a diagnostic challenge and causes high morbidity and mortality in children. Early suspicion of the disease and a rapid, safe and useful diagnostic test are relevant because up to 70% of the cases may die. We report the case of a newborn girl aged 25 days, who presented with a clinical picture that was compatible with herpes simplex encephalitis where the confirmation of the etiological diagnosis was delayed. Only by repeated real-time polymerase chain reaction it was possible to confirm the presence of herpes simplex virus type 1 in the cerebrospinal fluid.