SDC 1: Description of the Molluscum specific assay used in confirmatory diagnosis in case report.

The Molluscum specific assay was developed to target the poxvirus DNA polymerase gene, an essential gene conserved across the family of poxviruses; the probe and primer sequences of the assay were designed to be specific for Molluscum contagiosum based on available genome sequences: MOCV_probe (5’ FAM label, 3’ Blackhole quencher): 5’ACC GCC TCG ACG CCG AGA TC, MOCV_forward primer: 5’GGG TGG TGG CCA ACG, MOCV_reverse primer: 5’GCT TCG CAC AGC ACG TG. The qPCR assay was validated using clinical samples that had been demonstrated to contain Molluscum contagiosum and other available near-neighbor poxvirus DNAs using other laboratory methods. No cross reactions were observed for the Molluscum contagiosum virus (MOCV) specific qPCR assay using other high GC content poxvirus DNAs (data not show).

The DNA extracted from the clinical specimen was tested with MOCV qPCR assay described above using following conditions: Each reaction mixture contained 1X TaqMan Fast qPCR Master Mix (Applied Biosystems, Foster City, CA), 0.4 µmol/L each primer, 200 nmol/L of the FAM labeled TaqMan probe, and 2 µL of template DNA in a final reaction of 20 µL. Thermal cycling for the ABI Prism7900HT Sequence Detection System was: one cycle 95°C for 20 seconds; 45 cycles of 95°C for 1 second, and 60°C for 25 seconds. The MOCV qPCR presented a moderate amplification curve and crossed the threshold line (Ct) at 26.6, revealing presence of molluscum DNA in clinical specimen and confirmed MC infection.