Evaluation of published real-time PCR assays, designed to differentiate and identify variola virus, using a panel of non-variola orthopoxviruses

Meyer* H.¹ and A. Nitsche²
Bundeswehr Institute of Microbiology, Neuherbergstr. 11, 80937 München, Germany; ²Robert Koch-Institut, Nordufer 20, 13353 Berlin, Germany

The genus Orthopoxvirus, family Poxviridae, comprises morphologically and antigenically closely related species, including variola virus, the causative agent of smallpox. Variola virus is considered to be a potential threat agent or bioterrorist weapon. Various polymerase chain reaction (PCR) methods have been used to identify and subtype orthopoxviruses by using consensus primers combined with restriction cleavage. Today, real-time PCR is even more efficient because it combines amplification and detection of target DNA in one vessel, thereby eliminating any time-consuming follow up procedures potentially leading to cross-contamination.

In this study we evaluated published real-time PCR assays designed to specifically identify or discriminate variola virus with a panel of 100 non-variola orthopoxviruses. Two assays targeting the 14 kDa fusion protein gene (Olson et al., 2004; J Clin Microbiol 42:1940-6) and the rpo18 gene (Nitsche et al., 2004; J Clin Microbiol 42:1207-13) and relying on the concept of melting temperature analysis correctly discriminate all 100 non-variola orthopoxviruses from variola virus.

Two assays targeting the hemagglutinin gene (Espy et al., 2002, J Clin Microbiol 40:1985-8; Panning et al., 2004, Clin Chem 50:702-8) failed to discriminate variola virus from other orthopoxviruses because identical primer and probe sequences are present in some cowpox or camelpox virus strains.

Sequences of the hemagglutinin gene were also used by Ibrahim et al., 2003 (Clin Microbiol 41:3835-9) to design a variola virus-specific probe. This probe used as a minor groove binding protein version by Kulesh et al., 2004 (J Clin Microbiol 42:601-9) was indeed specific for variola virus although the assays at that time were evaluated with a single cowpox virus strain only.

Studies of two further variola-virus-specific assays (Kulesh et al., 2004; J Clin Microbiol 42:601-9) demonstrated that the primer and probe sequences used are conserved in some cowpox virus strains.

Here we demonstrated that especially with certain cowpox viruses false-positive “variola virus” amplifications occurred. Due to serious consequences of the diagnosis “smallpox” or the consequences of a misdiagnosis, we feel that more than one assay has to be applied in order to reliably identify smallpox.