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A Multiplex Real-time PCR Assay for the Rapid Detection of 4 Rash Causing Pathogens: Variola, Vaccinia, Orthopox and Varicella Zoster Viruses

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The perceived risk that smallpox could be used as a biological weapon has increased since the intentional release of anthrax in 2001. The development of a rapid detection method, able to distinguish between “look-a-like” diseases and the vaccine strain, is critical to ensure timely detection of a bioterror event. We have developed a single reaction, 4-target multiplex real-time PCR (rtPCR) assay which can discriminate between Varicella Zoster Virus (VZV, causative agent of chickenpox), variola (causative agent of smallpox), vaccinia (vaccine strain) and other Orthopox viruses (i.e.: monkeypox). The B10R, C8L, E9L and #28 genes were selected for detection of variola, vaccinia, Orthopox and VZV, respectively. Reporter dye chemistries, FAM, JOE, NED and ROX, were coupled with 3'-Black Hole Quenchers for analysis on the ABI Prism 7000 Sequence Detection System. The specificity of each target was tested against a panel of pathogens including normal wound flora, closely related organisms, and look-a-like diseases. Three suspect clinical rash specimens, 13 prairie dogs and two suspect human monkeypox cases have been evaluated using this multiplex assay. Two of the three suspect rash specimens were determined to be vaccinia positive based on the rtPCR results and were later confirmed using electron microscopy. The application of this multiplex assay in a clinical setting has demonstrated its usefulness as a rapid and sensitive method able to distinguish an intentional release of smallpox from a vaccination adverse event.

