Molecular Detection of Smallpox and Orthopox viruses

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Technology description

Description

The National Microbiology Laboratory (NML) has developed a kit that identifies orthopox virus and differentiates smallpox from monkeypox, camelpox, and vaccinia with a high degree of sensitivity. It contains primers, an amplification mixture that facilitates DNA strand growth, a positive control that confirms the test conditions are conducive to such growth, and synthetic templates that produce genetic material which corresponds to sequences of smallpox and monkeypox HA and cmB genetic material.

The NML kit relies on Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) to identify orthopox viruses. A double-stranded DNA helix can be separated longitudinally, and each of the strands that results can serve as a template for growth of a DNA strand identical to the one the template split from.

The NML kit primers are each designed to attach to a specific region of a separated DNA strand of orthopox virus, (either the HA region or the crmB region), and grow. If orthopox virus is present the appropriate primer will attach and grow a DNA strand.

The resulting genetic material is then treated with restriction enzymes, (Sau 3AI and Spe I in the case of the HA region, and Dra I, Alw 44 I, Ssp I, and Hpa I in the case of the crmB region), that interact with specific parts of the genetic material and break the strands into fragments. Analysis of the fragments created by the restriction enzymes identifies the strain of orthopox virus present.

The Challenge

Smallpox is a highly infectious, deadly disease caused by the variola virus, a member of the orthopox virus family. There is no effective treatment for smallpox, and the fatality rate for those not immunized against it is about thirty percent.

The smallpox virus is most commonly spread by droplets from the throat. Symptoms of smallpox, such as fever, headache, malaise, backache, and headache, usually appear within twelve to fourteen days of infection. A rash becomes visible about three days after the first appearance of symptoms. If the illness is fatal, death usually occurs five or six days after the rash appears.

The last reported smallpox case happened during October 1977 in Somalia, and the disease is now said to be eradicated. Vaccinations against smallpox ceased in North America and nearly all of Europe during the 1970’s, and during the 1980’s all vaccination against the disease ended.

The immunity produced by those vaccinations has been compromised by time. All samples of smallpox

were to have been destroyed or placed in repositories in the United States or Russia for storage, but concern has been expressed that samples may exist elsewhere, samples that could be used for bioterrorism.
Given the threat of bioterrorism, tools to fight smallpox are urgently needed. The ability to quickly identify smallpox infection is vital because measures to prevent the spread of smallpox must be taken quickly to avoid an epidemic. But, proper identification of smallpox in a medical setting can be difficult because smallpox can be mistaken for chickenpox and smallpox is nearly indistinguishable from monkeypox, another member of the orthopox family of viruses.
Many Orthopox virus family detection technologies do not differentiate between orthopox strains and many lack positive controls to show that test conditions are conducive to proper test function.
A reliable method of identifying orthopox virus and differentiating smallpox from monkeypox, camelpox, and vaccinia that incorporates a positive control is important for the prevention of a smallpox epidemic.
Smallpox detection technology is an important element of the defense against bioterrorism.
The National Microbiology Laboratory smallpox detection kit combines the ability to detect smallpox and differentiate it from other orthopox viruses with a positive control that verifies test conditions are adequate for proper test functioning. This robust technology would be a potent weapon in the fight against a smallpox epidemic.

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