NEWS RELEASE

U.S. Army Medical Research Institute of Infectious Diseases
Fort Detrick, Maryland

For release
January 29, 2004

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Smallpox in 50-Year-Old Tissues Detected by Integrated Diagnostics Approach: Molecular Detection of *Variola* Virus in Archival Pathology Specimen

A rare, preserved specimen of human tissue infected with *Variola* virus, the causative agent of smallpox, has given scientists the unique opportunity to test modern diagnostic capabilities for the virus.

Smallpox, a devastating disease, was eradicated in 1979 through the efforts of the World Health Organization (WHO). Currently, infectious *Variola* (a species of the genus *Orthopoxvirus*) is known to exist only in two WHO-sanctioned repositories, one in Russia and the other at the Centers for Disease Control and Prevention in Atlanta. However, there is concern that undisclosed reference stocks of the virus may exist—and given its potential as a biological threat agent, improved methods of detection and identification are a high priority.

According to an article published in this month's issue of Laboratory Investigation, the specimen used in the study was part of a private collection archived at the Indiana University School of Dentistry. The university investigation determined the specimen—an intact forearm and hand, presumably from a child—to be about 50 years old. It was offered to the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) for further analysis.

"This was a unique chance for us to test all the assays and techniques that we had been working on for a potential biothreat agent—and to do it using a real sample of a disease that no longer occurs," said lead author Randal J. Schoepp. The USAMRIID team included Michelle D. Morin, Mark J. Martinez, David A. Kulesh, Lisa Hensley, Thomas W. Geisbert, and Peter B. Jahrling, with Daniel R. Brady of Indiana University also contributing to the study.

According to Schoepp, working with the specimen was a challenge. It had been maintained under less-than-ideal storage conditions, exposed to extremes of temperature, and preserved in fixative for decades. Nonetheless, it afforded an unparalleled opportunity to test a modern, integrated diagnostic approach for smallpox.

The authors started with routine histology. Tissue samples were processed, embedded in paraffin blocks, sectioned, and mounted on slides for examination. The lesions present in these tissue samples were characteristic of poxvirus infection. Next, electron microscopy allowed the team to examine the structure of the virus. Despite the condition of the sample, this analysis readily revealed viral particles consistent with a poxvirus in various stages of replication.

Taken together, these results suggested primary smallpox disease. To add confidence to this presumptive diagnosis, the team extracted DNA from tissue sections and tested it using TaqMan ® assays. These assays are based on a process called polymerase chain reaction, or PCR, that can be monitored in real time.

Real-time PCR or TaqMan ® assays require just a fragment of genetic material that is amplified many times over to allow detection and identification of a biological agent. The technique is very sensitive and can tolerate small quantities of poor quality DNA, making it ideal for this study. The assay allowed the USAMRIID team to detect both orthopox and *Variola* viral DNA extracted from fixed tissue sections.

"This work is yet another example of the outstanding capabilities that reside at USAMRIID—from classical pathology methods to state-of-the-art molecular diagnostics," said Colonel Erik A. Henchal, commander of the Institute. "The combination of these tools into an integrated system, as envisioned by USAMRIID scientists, could change the way that biological agents are identified."

USAMRIID, located at Fort Detrick, Maryland, is the lead laboratory for the Medical Biological Defense Research Program, and plays a key role in national defense and in infectious disease research. The Institute's mission is to conduct basic and applied research on biological threats resulting in medical solutions (such as vaccines, drugs and diagnostics) to protect the warfighter. USAMRIID is a subordinate laboratory of the U.S. Army Medical Research and Materiel Command.

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