Project Title: Diagnosis of Biohazard Level 4 (reference laboratory)
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Executing Institute: Institute of Preventive Medicine National Defense Medical Center
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Abstract:

Recently the infectious diseases emerge commonly, and the frequency of travel and contact among people increase dramatically. Therefore, the possibility of that the infectious diseases befall in Taiwan follow increasingly. The biohazard level 4 viruses; such as Ebola hemorrhagic fever, Marburg fever, Lassa fever, Crimean-Congo hemorrhagic fever and Nipah viruses etc; belong to the high fatality of infectious diseases. Moreover they may spread through human-to-human and cause the panic. As to now, there is hardly any capability or experience to diagnose these biohazard level 4 viruses or some reemerging diseases in Taiwan. Hence, it is urgent to develop and establish a specific, sensitive, simple, speedy and reproducible diagnostic system, which can help the diagnoses during the early stage of epidemic and stop the further spread of the disease.

As a reference lab, we routinely collect the international and domestic epidemic reports and these correlated pathogenic bioinformatics. According as this information, the diagnoses of each pathogen that include molecular biological system, immunologic system, and viral isolation, will be set up. Therefore, the banks of primers and probes, DNA fragments, recombinant proteins, and polyclonal or monoclonal antibodies will be established and expanded. Once the outbreaks occur, we shall work in the biohazard level 4 equipments to protect the safety of workers. Once some epidemic outbreak, we will try to help the diagnosis of the sample in the biosafety level 4 laboratory.

During 2006, we received six samples from CDC of Taiwan, which were totally asked to check for B virus. All the results of real-time PCR, virus isolation and dot blot for antibody against B virus showed negative. Because the genes of B virus contain high GC content, it may hurt the PCR. Therefore, we synthesized a DNA fragment of Gg gene in B virus as the positive control to check the sensitivity of this real-time PCR. The result showed that this real-time PCR could detect as low as one copy. It supports that the sequence of the template matched with the primers should get positive. We also set up the real-time PCR systems to test small pox, Rift Valley hemorrhagic fever, Ebola and Marburg hemorrhagic fever viruses separately and synthesize the DNA fragments for the positive control. As for the traditional PCR, RT-PCR and nested PCR designed in the past will be rendered for further analysis of nucleotide sequence, because the DNA products of PCR or RT-PCR are longer. We also continued to work on the recombinant proteins and antibodies. Especially we extended the synthetic nucleocapsid genes of Lassa and Crimean Congo virus and changed the vectors. During this year, we put more effort on monoclonal antibodies work and there are several clones of monoclonal antibody against Lassa nucleocapsid protein. Now we continue to identify their epitopes.

Keyword: biohazard level 4 viruses, RT-PCR, specific antigen, expression and purification of recombinant proteins