Role of Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) as a Better Tool for Early Diagnosis of Human Rabies

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Background: For more than three millennia, rabies has been one of the most feared human diseases. An early confirmation of rabies is important for timely immunization and to prevent unnecessary post-exposure prophylaxis. The conventional viral antigen detection by direct immunofluorescence (DIF) and seller staining are less sensitive and the gold standard MIT however is laborious and time consuming. Thus, the present study was carried out i) to compare RT-PCR result with DIF and seller staining, and ii) to determine its role in early diagnosis of rabies.

Methods: Skin biopsies and corneal impression smears were collected ante mortem and brain tissue and CSF were collected at autopsy, from suspected rabies patients. Reverse transcriptase polymerase chain reaction (RT-PCR) was done to detect viral RNA in patients and infected mice tissues. To determine the earliest appearance of rabies virus RNA in the mouse tissues, fixed virus and clinical isolates were inoculated intracerebrally into mice. Two newborn mice per day were sacrificed at two day intervals from day 2 - 8 for fixed virus and day five till death in clinical isolates. Mice brain tissue and muzzle skins were subjected to DIF, Seller and RT-PCR

Results: Of 10 samples, six postmortem brain tissues were positive by RT-PCR followed by 5 by DIF and 4 for negri bodies. Rabies viral RNA could be detected as early as the 3rd day of infection by RTPCR in brain as well as muzzle tissues followed by rabies viral antigen by DIF on day 5th and symptoms on day 7 in fixed rabies virus infected mice, where as no qualitative difference was observed between 3 techniques when examined 5 days onwards.

Conclusion: The present study thus highlights the importance of RT-PCR as a more sensitive technique with the potentiality for early confirmation of rabies as compared to conventional techniques.

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Development of Serodiagnostic Method for the Detection of Serotype-Specific Anti-Coxsackievirus Antibodies

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Coxsackieviruses (CV) are associated with several clinical manifestations ranging from mild upper respiratory infections to meningitis and myocarditis. Different serotypes isolation and cultivation and the use of neutralization tests. In this study, we developed an Enzyme-Linked Immunosorbent Assay (ELISA) with the ability to discriminate among different serotypes of Coxsackie viruses.

Fifteen-mer peptides were synthesized in order to include the sequences where the antigenic protein VP1 differs among Coxsackie B3, B4 and A24 serotypes (pepB3, pepB4 and pepA24, respectively). The peptides were synthesized in conjunction with an oligopeptide carrier (MAP) and applied in the ELISA assay. In parallel, a commercial ELISA based on the VP1 protein of Coxsackieviruses B1/B5 as antigen was used. Sera were obtained from 200 healthy donors and examined for the presence of antibodies against pepB3, pepB4, pepA24 and VP1-B1/B5.

IgG antibodies against peptides of Coxsackie B3, B4 and A24 were detected at 23%, 33% and 36% of sera tested, respectively, while IgG antibodies against the VP1 of Coxsackie B1/B5 were found at 20,5% of the samples. Inhibition experiments demonstrated that (i) pepB3, pepB4 and pepA24 inhibited their homologous recognition by antibodies in ELISA at 57—87%, (ii) anti-pepA24 possess a distinct antigenic specificity, producing a heterologous inhibition of < 35% in anti-pepB3 and anti-pepB4 assays and (iii) anti-pepB3 and anti-pepB4 are distinct but their activities are related (demonstrating a heterologous inhibition of 8—68%, depending on serum, $r = 0,49$, $p < 0,0001$). Urea dissociation experiments demonstrated that anti-pepA24 antibodies can react specifically with high avidity with their peptide substrate, resisting even to the treatment of 8 M urea.

The peptides pepB3, pepB4 and pepA24 can be used for the detection of antibodies against Coxsackieviruses enabling the discrimination among different coxsackieviruse serotypes.

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Real-time Diagnosis and Monitoring of Cytomegalovirus Infections Using TaqMan-MGB Probe Technology

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Background: Cytomegalovirus (CMV) is an important pathogen in morbidity and mortality in immunocompromised patients including AIDS patients, infants, and transplant patients. In these patients, early detection and monitoring of CMV infection before clinically significant disease occurred may be useful for antiviral therapy and also allow detection of antiviral drug resistance. Today, nucleic acid detection assays are generally preferred over culture-based methods for detection of CMV infection. A real-time PCR assay based on highly conserved CMV glycoprotein B (gB) was developed to quantify the cytomegalovirus genome load.

Methods: CMV glycoprotein B containing plasmid was constructed by using the TOPO TA cloning procedures with plasmid pCRR2.1 (Invitrogen, Carlsbad, Calif.). The standard curve was established by plotting the CMV plasmid DNA input against the corresponding threshold cycle (Ct) values. TaqMan polymerase chain reaction assays based on CMV gB was