

## Molecular double check strategy for the identification and characterization of European Lyssaviruses

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## ABSTRACTS

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## D 22 - POSTER: Molecular double check strategy for the identification and characterization of European Lyssaviruses

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Lyssaviruses (order Mononegavirales, family Rhabdoviridae), the causative agents of rabies, represent a remarkable public health threat in developing countries. Among human exposures RABV is transmitted predominantly by dog bite; however bat lyssaviruses have also caused human cases. The “gold standard” for post-mortem rabies diagnosis is the fluorescence antibody test (FAT). However, in the case of ante-mortem non-neural sample material (e.g. saliva, cerebral spinal fluid, skin biopsies) or badly decomposed tissues the FAT reaches its limit and the use of molecular methods like reverse transcription PCR (RT-PCR) can be advantageous. In this study we developed a reverse transcription PCR cascade protocol feasible for screening and classification of samples even without any epidemiologic background with emphasis on the most relevant European lyssaviruses.

As a first step two independent pan-lyssavirus assays based on the detection of an intercalating dye are performed in a double check application to increase diagnostic safety. Additionally, two independent internal control systems (endogenous and heterologous) were established. For the second line characterization of the lyssavirus positive samples two independent probe based (TaqMan) species-specific multiplex systems for RABV, EBLV-1, EBLV-2 and BBLV were developed. All assays were successfully validated with a comprehensive panel of 52 lyssavirus positive samples (including RABV, LBV, MOKV, DUVV, EBLV-1 & -2, ABLV, BBLV) as well as negative material from various host species. Furthermore, a synthetic positive control for all assays (intercalating dye and TaqMan assays) was established which enables a quantification of the viral load.

In conclusion the developed pan-lyssavirus real-time RT-PCR assays and the two independent species-specific multiplex real-time RT-PCR systems allow the safe and sensitive screening and detection of all known lyssaviruses in humans and different animals as well as the characterization of the lyssavirus species circulating in the main land of Europe. The presented workflow combines all known advantages of the real-time PCR technology like speed and reduced risk of cross-contamination with improved safety of molecular testing based on double check strategy for the screening as well as the confirmation assays.