## EPIDEMIC SITUATION AND CONTROL MEASURES AGAINST EPIDEMIC HEMORRHAGIC FEVER AND WESTERN NILE FEVER IN THE REPUBLIC OF TATARSTAN

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*Within the years of 1997 to 2011 the epidemic hemorrhagic fever was registered in 42 regions and cities and 21440 cases were registered in human. Annually, the ratio of lethal cases is 0.8% from the infected patients. The principal source and the vector is a bank and common vole. In order to improve the epidemiological situation of the region the disinfestation along with personal and collective sanitation is promoted among the population.*

*In Tatarstan 4 cases of Western Nile fever infection were also registered. In this regard the prevention measures and informational works are performed.*

## VALIDATION OF COMMERCIAL REAL TIME RT-PCR KITS FOR DETECTION OF WEST NILE VIRUS

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The West Nile virus (WNV), also called West Nile virus is a flavivirus transmitted by mosquitoes. This virus can cause fatal neurological diseases in humans and horses (in about 1% of infections). Birds are the main reservoir hosts of the virus.

The monitoring of this virus by health authorities has helped to highlight an increase in severe forms of the disease, especially a worsening of nerve damage. Indeed, several human epidemics occurred in Europe, North America and around the Mediterranean. Also, several epidemics affecting only the horses have been described in Europe. The West Nile virus presents a significant variability of its stem: the stem lineage 1 is responsible for the vast majority of recent outbreaks and epidemics, lineage 2 is responsible for the epidemic and equine epizootic in Hungary in 2008. Therefore, it is necessary to develop adapted molecular tools to efficiently amplify a majority of strains of West Nile virus circulating in Europe, especially various strains on the nucleotide level (lineages 1, 2, 3 ...).

According to epidemiological data provided by the authorities, LSI wished to propose a kit for RT-PCR in real time to improve the detection of the genome of various strains of WNV, in collaboration with the French Afssa reference laboratory.

**Material & Methods.** kit has been developed from the work of the French reference laboratory for virus Nile fever (Afssa Lerpaz). The detection system was designed to detect a majority of strains of West Nile, particularly the strains involved in human and equine outbreaks that occurred in Europe in recent years.

The system was evaluated in silico and experimentally on strains of lineage 1 and 2.

The specificity of the kit was evaluated in silico and experimentally on a panel of West Nile virus strains and genetically related flaviviruses and also on viral strains responsible for equine encephalitis.

The diagnostic kit of WNV has been developed in duplex to allow the detection of the target and a control of the extraction and amplification of the target due to the presence of a IPC (Internal positive control) endogenously present in the native samples. It is also possible to validate the extraction of acellular matrices with a IPC exogenously added during the extraction of nucleic acids.

The characterization of rtRT-PCR West Nile (determination of the sensitivity, specificity, repeatability and reproducibility of the system) has helped to highlight the robustness of the kit.

RNA were amplified by a duplex rtRT-PCR assay in an ABI Prism 7500 Apparatus (Applied Biosystems) using (i) a set of primers and probe labelled with FAM reporter dye targeting the 3' non coding region of WNV, and (ii) a set of primers and probe labelled with VIC reporter dye targeting the BetaActin gene (internal positive control). The detection limit and efficiency of the rtRT-PCR was determined by testing a 10-fold dilution series of a WNV quantified RNA.

**Discussions & Conclusions.**The rtRT-PCR-N (LSI Taqvet WNV) is specific and highly sensitive. The detection limit was 6 copies per PCR demonstrating the ability of this assay to detect very few amounts of virus. The rtRT-PCR is able to detect the whole lineages of the west nile virus which allow the kit to be a good diagnosis tool for WN virus detection in case of outbreak or in case of epidemiological surveillance.

## ОЦІНКА КОМЕРЦІЙНОГО НАБОРУ ПЛР РЕАЛЬНОГО ЧАСУ ДЛЯ ВИЯВЛЕННЯ ВІРУСУ ЛИХОМАНКИ ЗАХІДНОГО НІЛУ

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*Вірус лихоманки Західного Нілу є флавівірусом, що передається комарами. Цей вірус може призвести до фатальних неврологічних захворювань у людей і коней (приблизно в 1% випадків). Птахи є основними резервуарами вірусу.*

*Моніторинг цього вірусу органами охорони здоров'я дозволив визначити зростання важких форм захворювання, особливо нервової форми. Кілька епідемій серед людей відбулися в Європі, Північній Америці і у всьому Середземномор'ї. Крім того, кілька епідемій, що вражали тільки коней були описані в Європі. Вірус лихоманки Західного Нілу проявляє значну варіабельність: штам лінії 1 відповідає за переважну більшість останніх спалахів та епідемій, штам лінії 2 несе відповідальність за епідемію і епізоотію коней в Угорщині в 2008 році. У зв'язку з цим необхідно розробити адаптовані молекулярні інструменти для ефективної ампліфікації більшості штамів вірусу лихоманки Західного Нілу, що циркулює в Європі.*

*LSI у співпраці з французької референт лабораторією AFSSA хотів би запропонувати набір для ПЛР у режимі реального часу для покращення виявлення генома різних штамів вірусу лихоманки Західного Нилу.*