Routine NAT-screening for West Nile Virus Infections in Germany: Being prepared

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IPFA / PEI 18\textsuperscript{th} international workshop on „Surveillance and screening of blood borne pathogens“
GFE NAT-screening proprietary System

*autoX 2.0*

GFE West Nile Virus PCR and HCMV PCR
GFE Blut mbH

Development, Manufacturing and Distribution of Assays and Devices for NAT Testing of Blood Donations
GFE Blut mbH

Fully automated Screening and Confirmation Testing
for
HCV; HIV-1 incl. -O,-N; HIV-2; HBV;
HAV; Parvovirus B19

autoX-System 2.0
The GFE autoX-System 2.0

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- Processes different sample types on the same platform
- Allows the use of different pool sizes
- Open to integrate new tests without changes in the platform design
- Scheduling
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GFE new Portfolio virus pipeline:

West Nile Virus

HCMV

Combinatorial screening for selected donor and recipient collectives
GFE new Portfolio virus pipeline:

West Nile Virus
West Nile is an insect-borne *flavivirus* commonly found in Africa, western Asia and the Middle East and the Western Hemisphere.

Zell and Wutzler, Solomon et al., 2003
West Nile Virus/2

- Lineage 1/Kunjin
- Lineage 1c (5)
- Lineage 2
- Lineage 3
- Lineage 4
  - Koutango
West Nile Virus has been detected in at least 48 species of mosquitoes, over 250 species of birds, and at least 18 mammalian species, including pets, horses and humans.

About 80% of WNV infections in humans undergo an asymptomatic course.

Approximately 20% develop West Nile fever, a febrile illness of sudden onset, often associated with a long recovery period.

Only a few cases (<0.2%) develop a neuroinvasive disease resulting in more serious symptoms, including meningitis or encephalitis, sometimes with fatal outcome.
- WNV has recently emerged as a major US public health concern due to the outbreak in the USA in 2002 with reported 4165 WNV cases (2946 cases with Encephalitis/Meningitis) and 284 deaths.

- The rapid spread throughout North America during the following years caused a number of blood transfusion- and organ transplant-associated transmission of WNV.

- Human viremia is low. Transfusion studies:<1-200 pfu/ml (100-80000 copies/ml). Average 40 pfu/ml (16000 copies/ml) (R. Lanciotti, Diagnostic and Reference Laboratory at the CDC)
West Nil Virus infections in Europe

- The largest outbreak of WNV infection in humans in the EU was recorded in Bucharest, Romania, in 1996–1997, when more than 500 cases were reported.

- Since 2008, several countries in Europe reported WNV activity due to different lineages (1 and 2).

- In 2010, Greece experienced a large outbreak with > 250 cases and 35 deaths.

- Both West Nile Virus Lineages 1 and 2 are present in Europe.
West Nil Virus infections in Europe

Epidemiological situation in 2011:

- The total number of WNV cases reported 2011 is lower than 2010 (96 in the EU).

- New cases were identified in Hungary and the former Yugoslav Republic of Macedonia.

Source: ECDC, 2012
Prevention and Risk minimizing measures regarding West Nile Virus transmission by blood products in Europe

2004:

- Deferral of Blood donations for 28 days from travellers from an area with ongoing transmission of WNV in humans (USA, Canada) during the seasonal period for West Nile Virus infection.*

- NAT testing of donations in the affected Areas.

2008: „West Nile Virus and Blood Product Safety in Germany“*

Antibody testing:
- 0.03% anti-WNV positives (14,437 donors tested)

RNA-testing:
- No WNV-positive RNA was detected (9,976 donors tested)

(*Pfleiderer et al., 2008)
NAT testing for WNV- infected blood products in Germany

Paul Ehrlich Institute, Germany
(2011)

Stufenplanverfahren:

Step 1: Survey regarding Implementation of risk-minimizing measures to prevent West Nile Virus transmission through Bloodproducts in Germany.

Criteria to be discussed:

- Deferral of Blood donations (european countries at risk)
- NAT-testing of minipools (< 16 donations/pool)
- Coverage of WNV lineages 1 and 2 with comparable sensitivity.
- Necessity, feasibility.

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Establishing a separate logistics and testing track for screening of seasonal and/or more specified donor or recipient groups.
Tests in the late phase of product development

GFE West Nile Virus PCR Kit
GFE West Nile Virus PCR Kit

GFE amplicon design strategy

- Collection and alignment of all available WNV Lineage 1 and 2 full-length sequences.
  - Primary amplicon definition.
  - Alignment of all available partial sequences of the pre-defined amplicon area
  - Cross check amplicon against lineages 3, 4, Koutango
- Primer/Probe design and in-silico analysis (Tm, secondary structure, etc.)

Test currently in development!
GFE West Nile Virus PCR Kit: Lineage detection

Lineage 1

Lineage 2

Lineage 3

Lineage 4

Lineage 5

Test currently in development!
Expected NAT-screening Specifications of the GFE West Nile Virus Kit:

- Flexible pool design (up to 16 donations per pool)
- 400 - 1 ml input per donation
- High sensitivity
- Non-competitive internal control
- Available for manual as well as fully automated processing (autoX-System)

Test currently in development!
Tests in the late phase of product development

GFE HCMV PCR Kit
Some facts:

-HCMV belongs to the family of the *Herpesviridae*.

- *Prevalence:* Varies widely (40-100%) and shows a dependency to the geographic and the socioeconomic status of the population.

- *HCMV infection:* Normally unnoticed in healthy people, potentially life threatening to immunocompromised people or newborn infants.

Measures to minimize the risk of Transfusion-mediated HCMV-transmission:

- Leucodepletion
- Antibody testing
- NAT-testing

Test currently in development!
Specifications of the HCMV Kit:

- Individual/pooled samples
- Input from 100 µl up to 1 ml per donation
- \( \text{LOD}_{95\%} \) per individual donation (pool of 8, 1 ml input per donation):
  \[ 70 \text{ IU/ml} \]
- Non-competitive internal control
- Available for manual as well as fully automated processing (autoX-System)

Test currently in development!
Processing different Pools and viral parameters on the *autoX*-Platform: Combined Nucleic acid extraction and NAT-analysis for West Nile Virus and HCMV.

WNV

- Pools (n x donations)

HCMV

- Pools (n x donations)

Nucleic acid extraction

PCR-setup

PCR-Analysis
Summary

Late stage GFE PCR Test developments:

- West Nile Virus
  - Robust detection of WNV Lineages 1-5.

- HCMV
  - Very good sensitivity and precision for pools of 8 donations.
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Both will be released soon!
Acknowledgements

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Thank you !!