West Nile virus (WNV) belongs to the family *Flaviviridae*. Two major genetic lineages are known: WNV lineage 1 (WNV-1) is prevalent in North America, parts of Africa, Europe, and Australia, whereas WNV lineage 2 (WNV-2) circulates in Southern Africa, and recently also in Europe. In general, infection in humans is asymptomatic or mild, but in < 1%, involvement of the central nervous system is associated with high mortality and long-term morbidity. WNV-2 was thought to be less virulent, but severe cases have been observed in Greece during a recent WNV-2 outbreak. Little is known about the dynamics of acute WNV-2 infection. In this study, the novel RealStar WNV RT-PCR kit was evaluated and viral concentrations in acute WNV-2 cases from Greece determined.

43 samples from 38 patients were collected in Greece in 2012. Samples were taken a median of 4 days after onset of symptoms. All serum and CSF samples were positive for WNV IgM antibodies, whereas 14/34 serum and 2/3 CSF samples were positive for WNV IgG antibodies.

All external quality assessment (EQA) samples were correctly identified using the RealStar WNV RT-PCR.

No cross-reactivity in samples containing flaviviruses other than WNV.

Highly sensitive RealStar WNV RT-PCR with a 95% limit of detection of 116.8 RNA copies/ml. Excellent correlation coefficient of 0.95 between RealStar WNV RT-PCR and expected external quality (EQA) assessment sample RNA concentrations.

Low WNV RNA concentrations in all PCR-positive samples. WNV RNA detectable in urine samples

• WNV-2 sequence was obtained from one urine sample (342-12)
• WNV RNA detectable in serum and urine, but not in cerebrospinal fluid
• WNV RNA detection in urine supports previous findings, that WNV is shed in urine
• WNV RNA detectable in 1/4 fatal cases only

The RealStar WNV RT-PCR kit proved to be a highly sensitive and specific tool, with even increased sensitivity in urine samples. Factors other than viral load may be involved in fatal cases, e.g. immune response or cytokine release. In conclusion, the novel kit will be a helpful tool for both diagnostic purposes and for research studies.