Evaluation of a new West Nile virus nucleic acid test for screening of blood donations

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BACKGROUND: West Nile virus (WNV) is transmitted to humans through mosquito bites and can be further transmitted to humans through transfusion or transplantation. Because most infected individuals are asymptomatic, blood donor screening is important in areas where WNV is endemic. These studies evaluated the performance of a new test for detection of WNV RNA in blood donations.

STUDY DESIGN AND METHODS: Analytical performance evaluation included sensitivity, specificity, inclusivity, and correlation. A clinical specificity study was conducted at four blood donor testing laboratories in parallel with the cobas TaqScreen WNV Test (Roche Molecular Systems, Inc.).

RESULTS: The 95% and 50% limit of detection for cobas WNV was 12.9 copies/mL (95% confidence interval [CI], 10.8–16.3) and 2.1 copies/mL (95% CI, 1.9–2.4) for WNV lineage 1, respectively, and 6.2 copies/mL (95% CI, 4.8–8.9) and 1.1 copies/mL (95% CI, 0.8–1.3) for WNV lineage 2, respectively. Clinical specificity was 100% in 10,823 donor samples tested individually (95% CI, 99.966%–100%) and 63,243 tested in pools of 6 (95% CI, 99.994%–100%). Samples of other members of the Japanese encephalitis virus serocomplex, including St Louis encephalitis, Japanese encephalitis, Murray Valley encephalitis, Usutu, and Kunjin viruses were detected by cobas WNV.

CONCLUSION: The cobas WNV test for use on the cobas 6800/8800 System, a fully automated test system, demonstrated high sensitivity and specificity and is suitable for the detection of WNV in blood donors.

West Nile virus (WNV) is an arbovirus that belongs to the Flaviviridae family, genus *Flavivirus,* and is a member of the Japanese encephalitis virus serocomplex.[1,2] The Japanese encephalitis serocomplex also includes Japanese encephalitis virus (JEV), St Louis encephalitis virus (SLEV), Murray Valley encephalitis virus (MVEV), Usutu virus (USUV),[3] and Kunjin virus (KUNV), now known to be a WNV variant.[4–6] WNV consists of several phylogenetic lineages;[7] however, only Lineage 1 (WNVL1) and Lineage 2 (WNVL2) have been associated with significant outbreaks in humans.[1,7] Due to the ability of WNV to infect numerous mosquito and bird species, the virus is distributed widely

ABBREVIATIONS: CI = confidence interval; JEV = Japanese encephalitis virus; KUNV = Kunjin virus; LoD = limit of detection; MVEV = Murray Valley encephalitis virus; NAT = nucleic acid testing; SLEV = St Louis encephalitis virus; USUV = Usutu virus; WNV = West Nile virus; WNVL1 = West Nile virus Lineage 1; WNVL2 = West Nile virus Lineage 2.

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The studies described in this manuscript were sponsored by Roche Molecular Systems, Inc.

Received for publication June 18, 2018; revision received September 7, 2018; and accepted September 22, 2018.

doi:10.1111/trf.15022

TRANSFUSION 2019;59;623–628

throughout the United States, Africa, the Middle East, southern Europe, Russia, Asia, and Australia.[1,8] Most WNV infections are asymptomatic; however, in some individuals, symptoms of infection can range from West Nile fever with a flulike illness to meningoencephalitis or even death.[1,9] In 2002, the first cases of transmission of WNV by blood transfusion were reported in the United States[8–12] and nucleic acid testing (NAT) of donors for WNV RNA was first implemented nationwide in 2003 using investigational assays.[13–15] Subsequently, the cobas TaqScreen West Nile Virus Test for use on the cobas s 201 system (Roche Molecular Systems, Inc.) was licensed by the US Food and Drug Administration for routine use in donor screening.

The cobas WNV test is a new assay for use on the fully automated cobas 6800/8800 Systems (Roche Molecular Systems, Inc.).[16] The aim of this paper is to describe the performance evaluation of cobas WNV.

MATERIALS AND METHODS

The cobas WNV test for use on the cobas 6800/8800 Systems is a qualitative in vitro test intended for use to screen blood donor samples for WNV RNA in plasma samples from donors of blood and blood components either as individual samples or in pools composed of aliquots of individual samples. The test can also be used to screen individual samples collected from living or cadaveric organ and tissue donors.[17]

The cobas WNV master mix contains detection probes that are specific for WNV and an internal control nucleic acid. The specific WNV and internal control detection probes are each labeled with one of two unique fluorescent dyes that act as a reporter. The two reporter dyes are measured at defined wavelengths permitting simultaneous detection and discrimination of the amplified WNV target and the internal control.[17]

The cobas 6800/8800 Systems provide fully automated sample preparation for nucleic acid extraction and purification with ready to use reagents, followed by polymerase chain reaction amplification and detection. Automated data management is performed by the System software, which assigns test results for all tests as nonreactive, reactive, or invalid. The cobas 6800/8800 Systems can process either pooled or individual specimens concurrently for cobas WNV and cobas MPX, a multiplex test for the detection of human immunodeficiency virus RNA, hepatitis C virus RNA, and hepatitis B virus DNA for donor blood screening. The high throughput of the cobas 6800/8800 Systems to process specimens simultaneously with multiple assays, the use of on-board universal reagents, and automated process control provide increased efficiency and an improved turnaround time compared to the cobas s 201 platform for TaqScreen assays.[16]

Nonclinical performance evaluation

Studies including evaluation of analytical sensitivity, analytical specificity, inclusivity, and correlation were performed at Roche Molecular Systems, Inc.

Analytical sensitivity

The limit of detection (LoD) for WNVL1 was determined using a secondary standard calibrated against the Health Canada WNV Reference Standard (Infectious Diseases, Canadian Blood Services, Ottawa, Ontario). The LoD for WNVL2 was determined using a WNVL2 isolate (ISS0513) provided by the National Centre for Immunobiologicals Research and Evaluation, Istituto Superiore di Sanita, Rome, Italy.

The WNVL1 Secondary Standard and WNVL2 viral isolate were each diluted in three different WNV negative human ethylenediaminetetraacetic acid (EDTA) plasma pools to generate three independent dilution series per lineage. Each panel was prepared by a unique operator and was prepared by diluting the Secondary Standard or viral isolate to the appropriate concentration above, below, and at the expected LoD, then aliquoted and stored frozen (−60 °C to −90 °C) until the day of testing. The panels were tested over multiple reagent lots, runs, days, operators, systems, and replicates per run. A total of 189 replicates were tested for each concentration of WNVL1 and a total of 72 replicates were tested for each concentration of WNVL2. The resulting data were analyzed to identify the 95% and 50% LoD based on probit analysis (SAS Biometric tool, SAS Institute Inc.).

Analytical specificity

Analytical specificity was determined by testing EDTA plasma specimens from 1000 healthy blood donors with cobas WNV using three different lots of reagents on three cobas 8800 Systems.

Cross reactivity was evaluated by testing 27 microorganisms at 1.00E+6 particles, copies, or PFU/mL, which included 20 viral isolates (including USUV), 6 bacterial strains, and 1 yeast isolate. The microorganisms were added to normal, WNV-negative human EDTA plasma and were tested in three replicates with and three replicates without WNV added to a concentration of approximately 3× LoD.

Patient plasma specimens representing 12 different disease states, listed in Table S1 (available as supporting information in the online version of this paper), were also tested with and without WNV viral target added to a concentration of approximately 3× LoD and were evaluated for sensitivity and specificity.

Inclusivity

Inclusivity for the cobas WNV test for EDTA-plasma was determined to ensure consistent detection of WNVL1, JEV, SLEV, MVEV, and KUNV. Dilutions of 10 WNVL1 cultured isolates were prepared by diluting cell culture supernatants in pooled WNV-negative EDTA-plasma to a concentration of

approximately 36 copies/mL. Each diluted isolate was tested once.

Serial dilutions of the cultured isolates for the flavivirus variants of WNV were prepared and tested. Two cultured isolates of JEV and one cultured isolate each of SLEV, MVEV, and KUNV were tested in four replicates in serial log dilutions prepared with pooled WNV negative plasma.

Correlation

The performance of the cobas WNV test and the cobas TaqScreen WNV Test was evaluated using 100 individual WNV NAT-positive samples and 100 individual WNV negative EDTA-plasma samples. The reported concentration range of the WNV-positive samples was 100 copies/mL to 85,000 copies/mL (SuperQuant WNV PCR Assay, National Genetics Institute). The WNV positive samples were tested neat and diluted 1:6. Correlation between cobas WNV test and cobas TaqScreen WNV test was assessed by calculating overall percentage agreement and performing McNemar's test (exact p value >0.05).[18]

Clinical performance evaluation

Clinical specificity

Four testing sites participated in a multicenter clinical study to determine the clinical specificity of cobas WNV for testing plasma from blood donor samples, both individually and in pools of up to six donations. Testing was performed under an Investigational New Drug application approved by the Food and Drug Administration. Each test site obtained approval from their institutional review board in accordance with Food and Drug Administration and local regulatory requirements before the start of the study.

The cobas p 680 instrument was used for creating pools of donor samples for cobas WNV testing. Three cobas WNV reagent lots were used for testing.

Each donation was tested by both cobas WNV on the cobas 6800/8800 Systems and by the site's routine test, the cobas TaqScreen WNV Test on the cobas s 201. Donations were tested either by individual donation testing or in pools of up to six donations by both tests. Reactive pools were resolved by testing the individual members of the pools. Results for a donation were considered evaluable if a valid result for both cobas WNV and cobas TaqScreen WNV Test was obtained by the same testing format (i.e., either individual donation testing or pools). Donors with discordant results between the two tests were to have additional testing performed on the index donation and to be invited to enroll in a follow-up study. A donation reactive on cobas WNV or the cobas TaqScreen WNV Test was defined as true positive for WNV if any of the following was true: (1) cobas WNV and the cobas TaqScreen WNV Test results were both reactive; (2) the index donation was positive for immunoglobulin M anti-WNV; (3) alternative licensed or validated NAT on the index plasma was positive; (4) donor was positive for immunoglobulin M anti-WNV on follow-up testing; or (5) the donor was positive for WNV NAT on follow-up testing.

Specificity was calculated as the frequency of cobas WNV nonreactive results among status-negative donations, defined as total donations minus true-positive donations.

Clinical sensitivity

Plasma samples from 530 US donations previously identified as WNV RNA reactive were obtained from frozen repositories; viral loads were reported to be 100 copies/mL or greater. Aliquots of the samples, both neat and diluted 1:6 (to simulate pools of six) were distributed across four testing sites and tested by cobas WNV. Nonreactive samples were retested by an alternative NAT at National Genetics Institute, Los Angeles, CA (NGI UltraQual 2X, and, if reactive, WNV quantitative polymerase chain reaction with a lower limit of quantification of 100 copies/mL).

RESULTS

Nonclinical performance evaluation

Analytical sensitivity

For WNVL1 and WNVL2, probit analysis on the data combined across dilution series and reagent lots was used to estimate the 95% and 50% LoD, along with the lower and upper limit of the 95% confidence interval (CI) (Table 1).

Analytical specificity

There were no reactive results with the 1000 EDTA-plasma samples from healthy donors; thus, the analytical specificity for cobas WNV was 100% (95% CI, 99.6–100%).

As shown in Table S2 (available as supporting information in the online version of this paper), nonreactive results without added WNV target were obtained for all samples containing microorganisms with the exception of USUV, and reactive results were obtained on all of the

TABLE 1. Analytical sensitivity by probit analysis

Analyte	Measuring units	Probit	LoD	Lower 95% confidence limit	Upper 95% confidence limit
WNV Lineage 1	Copies/mL	95% LoD	12.9	10.8	16.3
		50% LoD	2.1	1.9	2.4
WNV Lineage 2	Copies/mL	95% LoD	6.2	4.8	8.9
		50% LoD	1.1	0.8	1.3

LoD = limit of detection.

TABLE 2. Cultured isolates of WNV flavivirus variants

	% Reactive (reactive/valid replicates tested)			
Sample dilution	JEV	SLEV	MVEV	KUNV
1:1.00E+02	100% (8/8)	100% (4/4)	100% (4/4)	100% (4/4)
1:1.00E+03	100% (8/8)	100% (4/4)	100% (4/4)	100% (4/4)
1:1.00E+04	100% (8/8)	100% (4/4)	100% (4/4)	100% (4/4)
1:1.00E+05	100% (8/8)	100% (4/4)	100% (4/4)	100% (4/4)
1:1.00E+06	100% (8/8)	100% (4/4)	100% (4/4)	100% (4/4)
1:1.00E+07	100% (8/8)	100% (4/4)	100% (4/4)	100% (4/4)

JEV = Japanese encephalitis virus; SLEV = St. Louis encephalitis virus; MVEV = Murray Valley encephalitis virus; KUNV = Kunjin virus; WNV = West Nile virus.

microorganism samples to which WNV target had been added. Samples containing USUV were reactive in all replicates.

The cobas WNV test yielded nonreactive results for all of the disease state samples without added WNV target and reactive results for all of the disease state samples with added WNV target (Table S1 in supplemental information). The disease states did not interfere with the sensitivity or specificity of the cobas WNV test.

Inclusivity

All 10 WNVL1 diluted cell culture supernatants were reactive for WNV (100%). All replicates of sample dilutions for JEV, SLEV, MVEV, and KUNV were 100% reactive, as shown in Table 2.

Correlation

The cobas WNV test and the cobas TaqScreen WNV test demonstrated 100% specificity when testing 100 WNV-negative EDTA-plasma donors. Testing of 100 WNV-positive samples resulted in 100% overall percentage agreement when tested neat and 98% when tested diluted 1:6 (Table 3). One sample at 1:6 dilution was nonreactive with the cobas TaqScreen WNV Test and reactive with the cobas WNV test, and another sample at 1:6 dilution was nonreactive with cobas WNV and reactive with the cobas TaqScreen WNV Test. Viral loads for these two samples when tested neat were 200 copies/mL and 100 copies/mL, respectively.

Clinical performance evaluation

Clinical specificity

Among 74,066 donations tested, all were nonreactive on both the cobas WNV and the cobas TaqScreen WNV Test. The clinical specificity of cobas WNV in 10,823 donor samples tested individually was 100% (95% CI, 99.966%–100%). The clinical specificity of cobas WNV in 63,243 individual donations tested in pools of 6 was 100% (95% CI, 99.994%–100%) (Table 4).

Of 10,573 pools of 6 tested, there were no reactive pools.

Clinical sensitivity

Of the 530 WNV-positive archived donor samples, valid results were obtained for 528 specimens tested neat; 523 of the 528 (99.1%) were reactive on cobas WNV. Of the 5 samples that were nonreactive on neat testing, 3 were negative by alternative qualitative NAT, and 2 were reactive by the qualitative but not quantitative NAT. Of 530 samples tested diluted 1:6, valid results were obtained for all samples, and 510 (96.2%) were reactive on cobas WNV. The 20 samples nonreactive at 1:6 dilution were retested undiluted (neat) by alternative NAT: 4 samples were nonreactive on alternative qualitative NAT, 10 were reactive on the qualitative but not quantitative NAT, and 6 were detected on the quantitative assay with reported viral loads of 100 copies/mL (5 samples) or 200 copies/mL (1 sample).

TABLE 3. Correlation of positive samples

Methods		WNV results	
cobas TaqScreen WNV Test	cobas WNV	Neat	Diluted 1:6
Nonreactive	Nonreactive	0	0
Reactive	Nonreactive	0	1*
Nonreactive	Reactive	0	1†
Reactive	Reactive	100	98
Total		100	100
McNemar's test, p-value (two-sided, alpha = 0.05)		1.0	1.0

* Viral load for this sample when tested neat by National Genetic Institute using WNV RNA SuperQuant assay was 100 copies/mL.
† Viral load for this sample when tested neat by National Genetic Institute using WNV RNA SuperQuant assay was 200 copies/mL.

TABLE 4. Overall clinical specificity of the cobas WNV

Pool size	Frequency (n/N)*	Estimate in percent (95% exact CI)
Individual	10,823 / 10,823	100.000% (99.966%–100.000%)
Pools of 6	63,243 / 63,243	100.000% (99.994%–100.000%)
Overall	74,066 / 74,066	100.000% (99.995%–100.000%)

CI = confidence interval; WNV = West Nile virus.
*n/N = Number nonreactive/number of status negative donations.

DISCUSSION

WNV can be transmitted via transfused RBCs, platelets, and fresh frozen plasma, as well as through transplantation and perinatal exposure.[1,2,10,11] Transfusion-transmitted WNV usually occurs during the acute phase of infection, when infected individuals are viremic and asymptomatic but have not yet seroconverted.[12] Because few infected donors develop clinically significant disease, questioning blood donors for recent illness suggestive of WNV infection is ineffective at identifying infected/seropositive donors.[19–21] Data gathered from blood donor screening shows that extremely low-titer WNV viremia from very recently infected donors who have not yet developed WNV antibodies can efficiently transmit WNV infection.[10,22] Donations with very low viral loads have been implicated in cases of transfusion-related transmission of WNV,[10] which poses particular danger for immunocompromised patients, who are often the recipients of blood transfusions.[10,22] Although WNVL1 is the predominant lineage in North America, WNVL2 has been increasingly identified in Europe, with human outbreaks reported in Russia, central Europe, Greece, and Italy.[23] The cobas WNV test is highly sensitive for both WNVL1 and WNVL2, with the 95% and 50% LoD for WNVL1 at 12.9 and 2.1 copies/mL, and 6.2 and 1.1 copies/mL for WNVL2, respectively.

In addition, cobas WNV, like the cobas TaqScreen West Nile Virus Test, can detect other members of the Japanese encephalitis virus serocomplex, for example, JEV, SLEV, MVEV, KUNV, and USUV.[24,25] Although cobas WNV does not include a specific claim for the detection of USUV, a recent publication from Austria described the detection of six blood donors infected with USUV who were initially reactive with cobas WNV and upon further testing were found to be infected with USUV and not WNV.[26] USUV was first identified in Africa in 1959; epidemiologic studies since 2001 have demonstrated widespread prevalence in birds in Europe. The clinical consequences of USUV infection in humans are not well understood, but the virus has been associated with neuroinvasive illnesses in humans.[27,28] At this time, no cases of transfusion-transmitted USUV have been documented, although USUV is considered an emerging arbovirus due to the increased incidence of human infections.[25]

Other members of the JEV serocomplex have also been recently reported to have potential impact on blood transfusion safety. In Hong Kong, JEV was transmitted to a lung transplant recipient by an RBC unit from an asymptomatic blood donor, resulting in encephalitis and death in the recipient. JEV sequences in a sample from the donation were identical to those in the recipient.[29] In the United States, a case of SLEV neuroinvasive disease in a kidney transplant recipient was traced to an RBC unit from an asymptomatic blood donor who was demonstrated to have SLEV immunoglobulin M in a sample collected 77 days after donation.[30] Thus, it appears that other members of the JEV serocomplex may, like WNV, be present in the blood of asymptomatic donors and potentially cause significant disease in transfusion recipients. A WNV donor screening assay that also detects other members of the JEV serocomplex may therefore potentially have benefits beyond WNV detection.

The specificity of cobas WNV was outstanding: 100%. There were no reactive donations in in 10,823 donor samples tested individually or 63,243 donations tested in pools of 6. The specificity study was conducted from April to mid-June, prior to WNV season in the United States, when positive donations were not expected.

The ability of cobas WNV to detect positive specimens was verified through the correlation study involving 100 clinical and donor samples and the clinical study involving 530 archived US donor samples. In the correlation study, cobas WNV detected all WNV positive samples when tested neat, and all but one sample when tested at 1:6 dilution. In the clinical study, sensitivity was 99.1% in archived donor samples tested neat and 96.2% in the samples tested at 1:6 dilution. Although all of these previously frozen samples used for the clinical study had been reported to have viral loads of at least 100 copies/mL, the majority of the samples that were nonreactive in this study had viral loads that were no longer quantifiable when retested. It is not uncommon for viral loads to decrease during frozen storage. It should be noted, however, that low viral loads are common among WNV-positive blood donations; it has been reported that more than 50% of WNV positive donations in the United States are detectable only by individual donation testing.[31] Therefore, in the United States, it is recommended to convert from minipool screening to individual donation testing when WNV is active in the region.[32,33] The cobas WNV test is suitable for use in either format, with high sensitivity and 100% specificity demonstrated in these studies.

In summary, the cobas WNV assay, for use on the highly automated cobas 6800/8800 Systems, provides high sensitivity for detection of WNVL1 and WNVL2 and outstanding

specificity when used to screen donations either individually or in pools. The demonstrated detection by cobas WNV of samples of other viruses in the JEV serocomplex suggests the potential for additional benefits for blood safety.

CONFLICT OF INTEREST

JS, RB, NH, JRD, ND, LLP, and SAG are employees of Roche Molecular Systems, Inc. JPA, YE, DAW, and PCW were site principal investigators for the clinical specificity studies.

REFERENCES

1. Petersen LR, Brault AC, Nasci RS. West Nile virus: review of the literature. JAMA 2013;310:308-15.
2. Gray TJ, Webb CE. A review of the epidemiological and clinical aspects of West Nile virus. Int J Gen Med 2014;7:193-203.
3. Ashraf U, Ye J, Ruan X, et al. Usutu virus: an emerging flavivirus in Europe. Viruses 2015;7:219-38.
4. Kuno G, Chang G-JJ, Tsuchiya KR, et al. Phylogeny of the genus *Flavivirus*. J Virol 1998;72:73-83.
5. Burke DS, Monath TP. Flaviviruses. In: Knipe DM , editors. Fields virology. Philadelphia, PA: Lippincott Williams & Wilkins; 2001. p. 1043-126.
6. Mackenzie JS, Gubler DJ, Petersen LR. Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. Nat Med 2004;10:S98-109.
7. May FJ, Davis CT, Tesh RB, et al. Phylogeography of West Nile virus: from the cradle of evolution in Africa to Eurasia, Australia, and the Americas. J Virol 2011;85:2964-74.
8. Petersen LR, Hayes EB. West Nile Virus in the Americas. Med Clin North Am 2008;92:1307-22.
9. Hollinger FB, Kleinman S. Transfusion transmission of West Nile virus: a merging of historical and contemporary perspectives. Transfusion 2003;43:992-7.
10. Pealer LN, Marfin AA, Petersen LR, et al. Transmission of West Nile Virus through blood transfusion in the United States in 2002. N Engl J Med 2003;349:1236-45.
11. Harrington T, Kuehnert MJ, Kamel H, et al. West Nile virus infection transmitted by blood transfusion. Transfusion 2003;43:1018-22.
12. Biggerstaff BJ, Petersen LR. Estimated risk of West Nile virus transmission through blood transfusion during an epidemic in Queens, New York City. Transfusion 2002;42:1019-26.
13. Epstein JS. Insights on donor screening for West Nile virus. Transfusion 2005;45:460-2.
14. Petersen LR, Epstein JS. Problem solved? West Nile Virus and transfusion safety. N Engl J Med 2005;353:516-7.
15. Busch MP, Wright DJ, Custer B, et al. West Nile Virus infections projected from blood donor screening data, United States, 2003. Emerg Infect Dis 2006;12:395-402.
16. Cobb B, Simon CO, Stramer SL, et al. The cobas® 6800/8800 system: a new era of automation in molecular diagnostics. Expert Rev Mol Diagn 2017;17:167-80.
17. cobas® WNV nucleic acid test for use on the cobas® 6800/8800 systems, 07175531001-03EN.
18. McNemar Q. Note on the sampling error of the difference between correleated proportions or percentages. Psychometrika 1947;12:153-7.
19. Custer B, Kamel H, Kiely NE, et al. Associations between West Nile virus infection and symptoms reported by blood donors identified through nucleic acid test screening. Transfusion 2009;49:278-88.
20. Petersen LR, Busch MP. Transfusion-transmitted arboviruses. Vox Sang 2010;98:495-503.
21. Orton SL, Stramer SL, Dodd RY. Self-reported symptoms associated with West Nile virus infection in RNA-positive blood donors. Transfusion 2006;46:272-7.
22. Centers for Disease Control and Prevention (CDC). Fatal West Nile Virus infection after probable transfusion-associated transmission colorado. MMWR Morb Mortal Wkly Rep 2013;62:622-4.
23. Hernandez-Triana LM, Jeffries CL, Mansfield KL, et al. Emergence of West Nile virus lineage 2 in Europe: a review on the introduction and spread of a mosquito-borne disease. Front Public Health 2014;2:271.
24. cobas(R) TaqScreen West Nile test for use on the cobas s 201system, 04741692001-12EN.
25. Cadar D, Maier P, Muller S, et al. Blood donor screening for West Nile virus (WNV) revealed acute Usutu virus (USUV) infection, Germany, September 2016. Euro Surveill 2017;22(14): 30501.
26. Bakonyi T, Jungbauer C, Aberle SW, et al. Usutu virus infections among blood donors, Austria, July and August 2017 – raising awareness for diagnostic challenges. Euro Surveill 2017;22(41): 17-00644.
27. Gaibani P, Rossini G. An overview of Usutu virus. Microbes Infect 2017;19:382-7.
28. Sejvar JJ, Haddad MB, Tierney BC, et al. Neurologic manifestations and outcome of West Nile virus infection. JAMA 2003; 290:511-5.
29. Cheng VCC, Sridhar S, Wong SC, et al. Japanese encephalitis virus transmitted via blood transfusion, Hong Kong, China. Emerg Infect Dis 2018;24:49-57.
30. Venkat H, Adams L, Sunenshine R, et al. St. Louis encephalitis virus possibly transmitted through blood transfusion–Arizona, 2015. Transfusion 2017;57:2987-94.
31. Dodd RY, Foster GA, Stramer SL. Keeping blood transfusion safe from West Nile virus: American red cross experience, 2003 to 2012. Transfus Med Rev 2015;29:153-61.
32. AABB. Association bulletin #13–02 – West Nile Virus nucleic acid testing – revised recommendations. Bethesda, MD; 2013.
33. US Food and Drug Administration. Guidance for industry: use of nucleic acid tests to reduce the risk of transmission of West Nile Virus from donors of whole blood and blood components intended for transfusion. Silver Spring, MD: CBER Office of Communication, Outreach, and Development; 2009.