Rx Only

cobas® WNV

Nucleic acid test
for use on the cobas® 6800/8800 Systems

For in vitro diagnostic use

IVD

cobas® WNV – 96	P/N: 07001061190
cobas® WNV – 480	P/N: 07001070190
cobas® WNV Control Kit	P/N: 07001118190
cobas® NHP Negative Control Kit	P/N: 07002220190
cobas omni MGP Reagent	P/N: 06997546190
cobas omni Specimen Diluent	P/N: 06997511190
cobas omni Lysis Reagent	P/N: 06997538190
cobas omni Wash Reagent	P/N: 06997503190

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Intended use

cobas® WNV for use on **cobas**® 6800 and **cobas**® 8800 Systems is a qualitative in vitro nucleic acid screening test for the direct detection of West Nile virus (WNV) RNA in human plasma.

This test is intended for use to screen donor samples for WNV RNA in plasma samples from individual human donors, including donors of whole blood and blood components, as well as other living donors. This test is also intended for use to screen organ and tissue donors when donor samples are obtained while the donor's heart is still beating using plasma or from cadaveric (non-heart beating) donors using plasma and serum.

Plasma from all donors may be screened as individual samples. For donations of whole blood and blood components, plasma samples may be tested individually or in pools comprised of not more than six individual samples. For all other donors, samples may only be screened as individual samples.

For donations from cadaveric (non-heart-beating) organ and tissue donors, samples may only be screened as individual samples.

This test is not intended for use as an aid in diagnosis of WNV infection.

This test is not intended for use on samples of cord blood.

Summary and explanation of the test

Background: Screening of blood for transfusion-transmitted viral infections

West Nile virus (WNV) is a single-stranded, positive-sense, arthropod-borne (arbovirus) RNA virus that belongs to the *Flaviviridae* family, genus *Flavivirus*, and the Japanese encephalitis virus serocomplex.[1,2] The Japanese encephalitis serocomplex also includes Japanese encephalitis virus, St. Louis encephalitis virus, Murray Valley encephalitis virus, and Kunjin virus (now known to be a WNV variant).[3-5] Phylogenetic studies have identified 2 main lineages of WNV: lineage 1 and lineage 2. Strains from lineage 1 are found in Africa, India, Australia, and the Western Hemisphere and have been responsible for recent epidemics in Europe, the Mediterranean basin, and the Americas. Strains from lineage 2 have been reported in sub-Saharan Africa[6] and more recently Southern Europe.[7,8]

Like other arboviruses, WNV is maintained in an enzootic cycle between blood-feeding mosquitoes and susceptible vertebrate hosts (birds).[9] Birds serve as the natural reservoir vertebrate host and mosquitoes of the genus *Culex* are the principal enzootic vectors for WNV, while humans and mammals (e.g., horses) are incidental and, usually, dead-end hosts because they rarely develop viremia of sufficient titer to infect efficiently arthropod vectors.[2,9,10]

WNV is distributed widely throughout Africa, the Middle East, southern Europe, western Russia, southwestern Asia, and Australia (Kunjin subtype of WNV), because of WNV's ability to infect numerous mosquito and bird species.[1] Human outbreaks, mainly associated with mild febrile illnesses, were reported infrequently in Israel and Africa until the mid-1990s.[1] Since the mid-1990s, new viral strains, likely with African origins, have resulted in increased numbers of infections in parts of Russia and southern and Eastern Europe, with large outbreaks of increased clinical severity occurring in Romania, Russia, Israel, and Greece.[1,8,11] WNV now circulates in many countries in the Western Hemisphere, but only the United States and Canada have experienced substantial human disease incidence.[1,12]

WNV first emerged in the United States in 1999 in New York City and spread rapidly across the entire United States in subsequent years.[13] WNV is now endemic in all 48 contiguous United States, as well as all Canadian provinces.[1] WNV has produced the three largest arboviral neuroinvasive disease (encephalitis, meningitis, or acute flaccid paralysis) outbreaks ever recorded in the United States, with nearly 3,000 cases of neuroinvasive disease recorded each

year in 2002, 2003, and 2012.[1] High viral activity occurs during the warm months of the year.[1] 94% of patients with WNV infection develop symptom onset in the summer months.[1]

WNV is estimated to have infected more than 4 million people in the United States between 1999 and 2012,[1] with a reported total of 16,196 patients with WNV neuroinvasive disease, including 1,549 related deaths.[1]

Rationale for NAT testing

WNV was first shown to be transmissible by transfusion and organ transplantation during investigations of an epidemic in the United States in 2002.[14,15] WNV can be transmitted via transfused red blood cells, platelets, fresh frozen plasma, and heart, kidney, liver, and lung transplants, although mosquito bites cause most WNV infections in humans.[1,2,14,16] WNV can also potentially be transmitted through hematopoietic progenitor cell transplantation. Transplacental and perinatal transmission of WNV has been reported.[1] Breast milk transmission, patients undergoing kidney dialysis, and occupational exposure (e.g., laboratory workers [percutaneous or conjunctival exposure]; poultry farm workers) are other rare modes of WNV transmission.[1,12] Infection usually produces lifelong immunity.[9]

Transfusion-transmitted WNV usually occurs during the acute phase of infection, when infected individuals are viremic and asymptomatic but have not yet seroconverted.[17] Since few infected donors develop clinically-significant disease, questioning blood donors for recent illness suggestive of WNV infection is ineffective at identifying infected/seropositive donors.[18,19] 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 efficiently transmit WNV infection.[9,20] Donations with very low viral loads have been implicated in cases of transfusion-related transmission of WNV,[21] which poses particular danger for immunocompromised patients, who are the recipients of the majority of blood transfusions.[22]

Nationwide nucleic acid testing (NAT) for WNV RNA was implemented in 2003 to insure transfusion safety.[9] During the first 2 years of WNV NAT screening of blood donations in the United States, 1,039 positive donors were identified among 27.2 million donations (0.4 per 10,000 donations), but the numbers ranged as high as 1 in 150 donors in some areas during epidemics.[18] NAT screening of blood donations in the United States and Canada has nearly eliminated the risk of transfusion-transmitted West Nile virus infection.[1] Between 2003 and 2013, approximately 3,000 WNV infections were interdicted.[23]

Among persons who become infected with WNV, approximately 80% are asymptomatic, 20% to 25% develop West Nile fever,[1,24] and 1 in 150 to 250 develop neuroinvasive disease.[1,25] West Nile fever consists of sudden onset headaches, malaise, fever (usually low grade), myalgia, chills, vomiting and other gastrointestinal symptoms, rash, fatigue, and eye pain, which can last a few days to a few weeks or even months.[1,24] West Nile neuroinvasive disease can manifest as meningitis, encephalitis, meningoencephalitis, or acute flaccid paralysis, which can lead to irreversible neurological damage, coma, and death.[1,26-31] WNV infection is also associated with myocarditis, pancreatitis, fulminant hepatitis, rhabdomyolysis, multifocal choroiditis, vitritis, and autonomic instability.[1]

The sequelae of neuroinvasive disease can persist for months to years after recovery from acute infection. After discharge from the hospital, individuals with West Nile encephalitis often require assistance with activities of daily living.[1,31,32] Neuropsychiatric symptoms, including depression and anxiety, as well as neurocognitive deficits, may persist for months to a year or longer.[1,20,33] About 10% of individuals who develop neuroinvasive West Nile disease die as a result; advanced age is the most important risk factor.[2] The risk of fatality is 17% for patients age 70 years or older, compared to a 0.8% risk of death for patients younger than 40 years of age.[1,33] Other risk factors for death include encephalitis with severe muscle weakness, altered level of consciousness, diabetes, cardiovascular disease, hepatitis C virus infection, and immunosuppression.[1,12,33]

Explanation of the test

cobas® WNV is a qualitative test that is run on the **cobas**® 6800 System and **cobas**® 8800 System. **cobas**® WNV enables the simultaneous detection of WNV RNA and the internal control in a single test of an infected, individual donation or pooled plasma from individual donations.

Principles of the procedure

cobas® WNV is based on real time PCR technology on a fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection system. The **cobas**® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas**® 6800/8800 software which assigns test results for all tests as non-reactive, reactive, or invalid. Results can be reviewed directly on the system screen, and printed as a report or sent to a Laboratory Information Management System (LIMS) or other result management system.

Samples can either be tested individually or, optionally, can be tested in pools consisting of multiple samples. The **cobas p** 680 instrument or **cobas**® **Synergy** software with the Hamilton MICROLAB® STAR IVD (**cobas**® **Synergy** Core), may optionally be used in a pre-analytical step if pooling is to be performed.

Nucleic acid from the sample and added armored RNA internal control (IC) (which serves as the sample preparation and amplification/detection process control) are simultaneously extracted. In addition the test utilizes two kit controls: a positive and a negative control. Viral nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris, and potential PCR inhibitors (such as hemoglobin) are removed with subsequent wash reagent steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.

Selective amplification of target nucleic acid from the donor sample is achieved by the use of virus-specific forward and reverse primers which are selected from highly conserved regions of the viral nucleic acid. A thermostable DNA polymerase enzyme is used for both reverse-transcription and amplification. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).[34-36] Any contaminating amplicon from previous PCR runs are destroyed by the AmpErase enzyme [uracil-N-glycosylase], which is included in the PCR mix, when heated in the first thermal cycling step. However, newly formed amplicon are not destroyed since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

cobas® WNV master mix contains detection probes which are specific for WNV and IC nucleic acid. The specific WNV and IC detection probes are each labeled with one of two unique fluorescent dyes which act as a reporter. Each probe also has a second dye which acts as a quencher. The two reporter dyes are measured at defined wavelengths, thus permitting simultaneous detection and discrimination of the amplified WNV target and the IC.[37,38] When not bound to the target sequence, the fluorescent signal of the intact probes is suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage by the 5' to 3' nuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye is concomitantly increased. Since the two specific reporter dyes are measured at defined wavelengths, simultaneous detection and discrimination of the amplified WNV target and the IC are possible.

Reagents and materials

cobas® WNV reagents and controls

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 4.

Table 1 cobas® WNV

Store at 2-8°C
96 test cassette (P/N 07001061190)
480 test cassette (P/N 07001070190)

Kit components	Reagent ingredients	Quantity per kit 96 tests	Quantity per kit 480 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin. May produce an allergic reaction.	13 mL	38 mL
Internal Control (IC)	Tris buffer, < 0.05% EDTA, < 0.001% internal control armored RNA construct (non-infectious RNA encapsulated in MS2 bacteriophage), < 0.002% Poly rA RNA (synthetic), < 0.1% sodium azide	13 mL	38 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	13 mL	38 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	5.5 mL	14.5 mL
WNV Master Mix Reagent 2 (WNV MMX-R2)	Tricine buffer, potassium acetate, glycerol, 18% dimethyl sulfoxide, Tween 20, EDTA, < 0.06% dATP, dGTP, dCTP, < 0.14% dUTP, < 0.01% upstream and downstream WNV and internal control primers, < 0.01% fluorescent-labeled WNV probes, < 0.01% fluorescent-labeled internal control probe, < 0.01% oligonucleotide aptamer, < 0.01% Z05D DNA polymerase, < 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	6 mL	17.5 mL

Table 2 cobas® WNV Control Kit

Store at 2-8°C
(P/N 07001118190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
WNV Positive Control (WNV (+) C)	< 0.001% Synthetic (armored) WNV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, WNV RNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	16 mL (16 x 1 mL)	  **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing dust/ fumes/gas/ mist/ vapours/ spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1)

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

Table 3 cobas® NHP Negative Control Kit

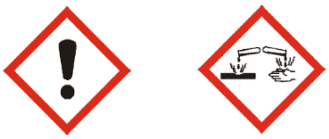
Store at 2-8°C
(P/N 07002220190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
Normal Human Plasma Negative Control (NHP-NC)	Normal human plasma, WNV RNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative**	16 mL (16 x 1 mL)	  **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 Mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1)

* Product safety labeling primarily follows EU GHS guidance
**Hazardous substance

cobas omni reagents for sample preparation

Table 4 cobas omni reagents for sample preparation*

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	42.56% (w/w) guanidine thiocyanate***, 5% (w/v) polydocanol***, 2% (w/v) dithiothreitol***, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302+ H332: Harmful if swallowed or if inhaled. H314: Causes severe skin burns and eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P273: Avoid release to the environment. P280: Wear protective gloves/protective clothing/eye protection/face protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor P305 + P351 + P338+ P310: IF IN EYES Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

* These reagents are not included in the **cobas**® WNV test kit. See listing of additional materials required (Table 7).

** Product safety labeling primarily follows EU GHS guidance

***Hazardous substance

Reagent storage and handling requirements

Opened reagents shall be stored and will be handled as specified in Table 5 and Table 6.

When reagents are not loaded on the **cobas**® 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

Table 5 Reagent storage (when reagent is not on the system)

Reagent	Storage temperature
cobas® WNV – 96	2–8°C
cobas® WNV – 480	2–8°C
cobas® WNV Control Kit	2–8°C
cobas® NHP Negative Control Kit	2–8°C
cobas omni Lysis Reagent	2–8°C
cobas omni MGP Reagent	2–8°C
cobas omni Specimen Diluent	2–8°C
cobas omni Wash Reagent	15–30°C

Reagents loaded onto the **cobas**® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The system allows reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

Table 6 Reagent expiry conditions enforced by the **cobas**® 6800/8800 Systems

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® WNV – 96	Date not passed	30 days from first usage	Max 10 runs	Max 8 hours
cobas® WNV – 480	Date not passed	30 days from first usage	Max 20 runs	Max 20 hours
cobas® WNV Control Kit	Date not passed	Not applicable	Not applicable	Max 10 hours
cobas® NHP Negative Control Kit	Date not passed	Not applicable	Not applicable	Max 10 hours
cobas omni Lysis Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days from loading*	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days from loading*	Not applicable	Not applicable

* Time is measured from the first time that reagent is loaded onto the **cobas**® 6800/8800 Systems.

Additional materials required

Table 7 Material and consumables for use on **cobas**® 6800/8800 Systems

Material	P/N
cobas omni Processing Plate	05534917001
cobas omni Amplification Plate	05534941001
cobas omni Pipette Tips	05534925001
cobas omni Liquid Waste Container	07094388001
cobas omni Lysis Reagent	06997538190
cobas omni MGP Reagent	06997546190
cobas omni Specimen Diluent	06997511190
cobas omni Wash Reagent	06997503190
Solid Waste Bag	07435967001
Solid Waste Container	07094361001

Instrumentation and software required

The **cobas**® 6800/8800 software and **cobas**® WNV analysis package shall be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system. The **cobas**® **Synergy** software shall be installed, if applicable.

Table 8 Instrumentation

Equipment	P/N
cobas® 6800 System (Option Moveable)	05524245001 and 06379672001
cobas® 6800 System (Fix)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001
Options for pipetting and pooling	**P/N**
cobas p 680 Instrument	06570577001
cobas® **Synergy** software Dongle	07788339001
Hamilton MICROLAB® STAR IVD	04640535001

Refer to the **cobas**® 6800/8800 Systems Operator's Manual and **cobas** p 680 instrument Operator's Manual or to the **cobas**® Synergy software User Assistance for additional information about primary and secondary sample tubes accepted on the instruments.

Note: Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For in vitro diagnostic use only.
- All samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[39,40] Only personnel proficient in handling infectious materials and the use of **cobas**® WNV, **cobas**® 6800/8800 Systems and optionally **cobas p** 680 instrument or the Hamilton MICROLAB® STAR IVD with **cobas**® **Synergy** Core should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- **cobas**® WNV Control Kit and **cobas**® NHP Negative Control Kit contain plasma derived from human blood. Testing of normal human plasma by PCR methods also showed no detectable WNV RNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
- Do not freeze whole blood.
- The use of sterile disposable pipettes and nuclease-free pipette tips is recommended. Use only supplied or specified required consumables to ensure optimal test performance.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- **cobas**® WNV kits, **cobas omni** MGP Reagent, and **cobas omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® WNV kits and **cobas omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls. Change gloves if contaminated by sample, control, or reagents.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 6800/8800 instruments, follow the instructions in the **cobas**® 6800/8800 Systems Operator's Manual to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport, storage, and pooling

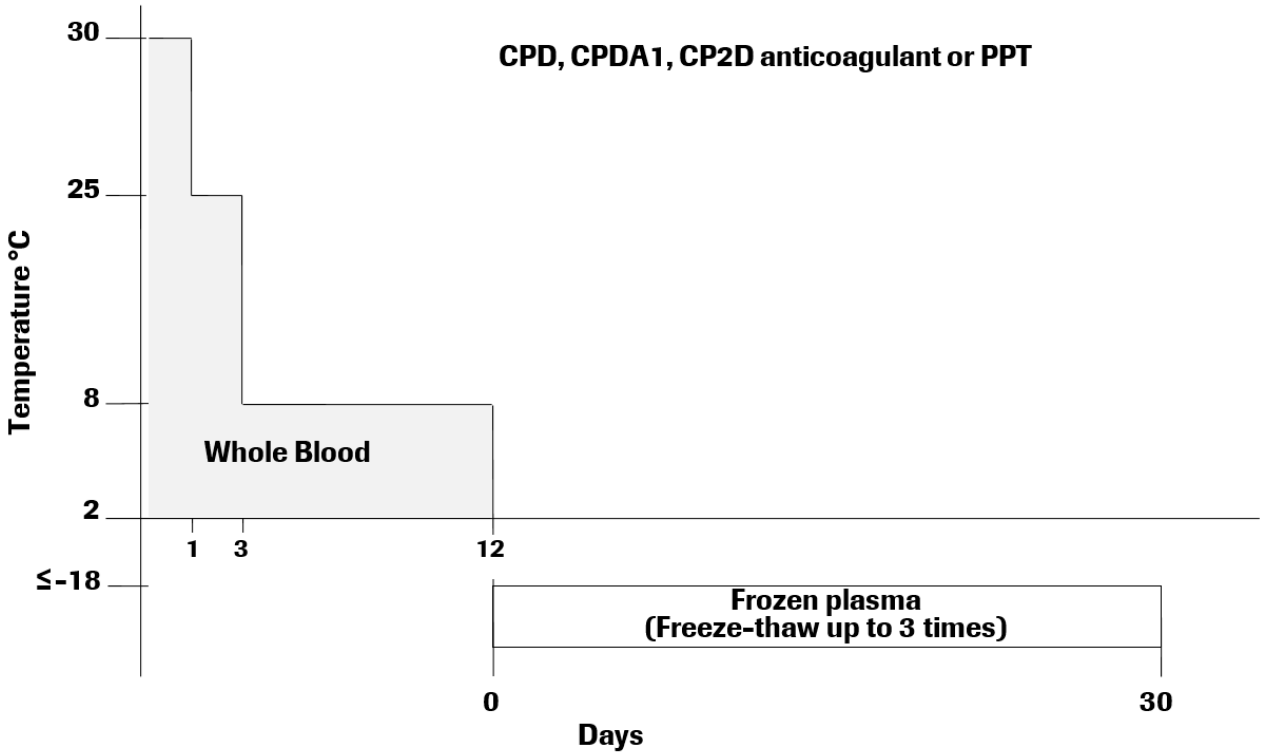
Note: Handle all samples and controls as if they are capable of transmitting infectious agents.
Store all donor samples at specified temperatures.
Sample stability is affected by elevated temperatures.

Living donor blood samples

- Plasma collected in EDTA, CPD, CPDA1, CP2D anticoagulant may be used with **cobas**® WNV. Follow the sample collection tube/bag manufacturer instructions for handling and centrifugation.
- Blood collected in CPD, CPDA1 or CP2D anticoagulant or Becton-Dickinson EDTA Plasma Preparation Tubes (BD PPT™) may be stored for up to 12 days with the following conditions:
  - Samples must be centrifuged within 72 hours of draw.
  - For storage above 8°C, samples may be stored for 72 hours at up to 25°C, and up to 30°C for 24 hours during the 72 hours.

Other than noted above, samples are stored at 2-8°C. In addition, plasma separated from the cells may be stored for up to 30 days at ≤-18°C with three freeze/thaw cycles. Refer to Figure 1.

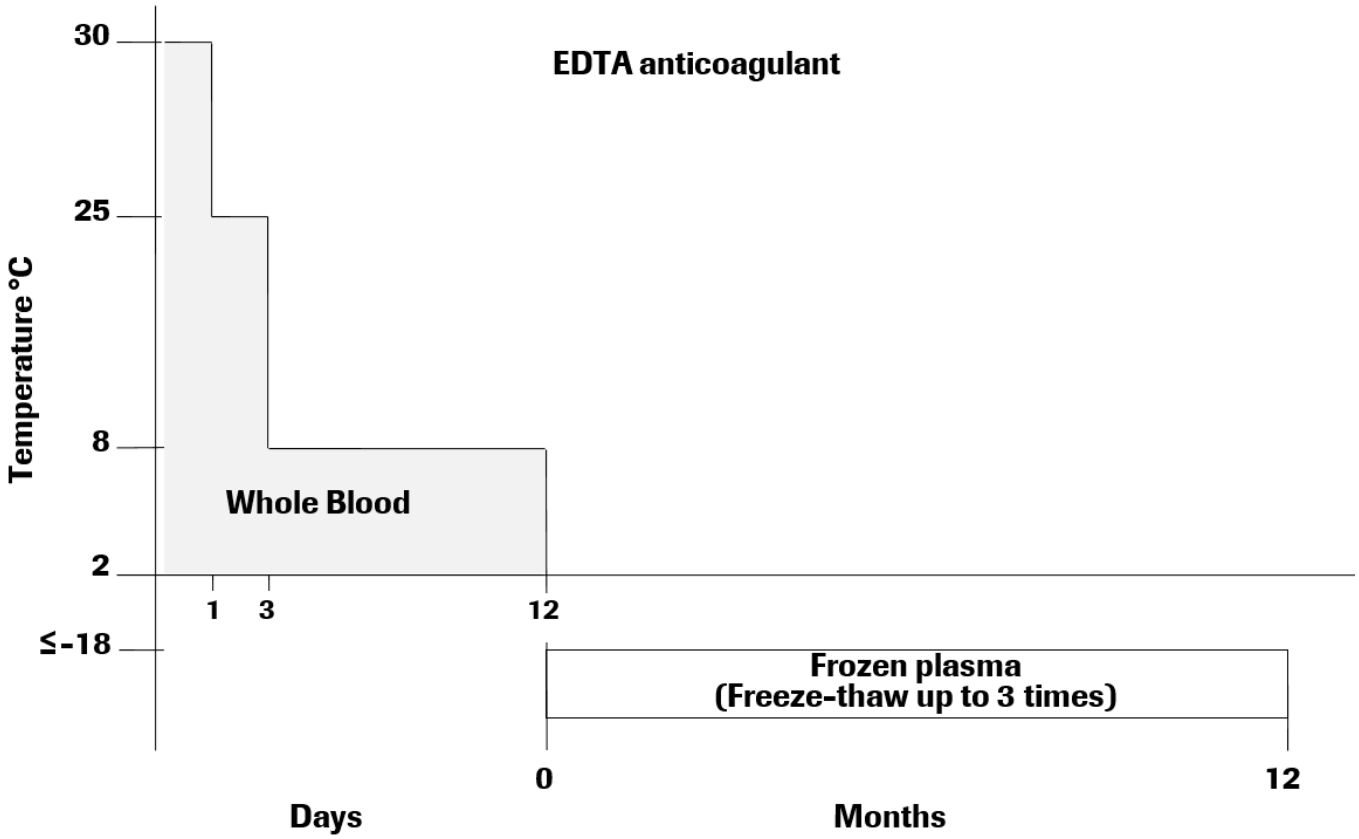
Figure 1 Sample storage conditions for living donor sample



- Blood collected in EDTA anticoagulant may be stored for up to 12 days with the following conditions:
  - Samples must be centrifuged within 72 hours of draw.
  - For storage above 8°C, specimens may be stored for 72 hours at up to 25°C, and up to 30°C for 24 hours during the 72 hours.

Other than noted above, samples are stored at 2-8°C. In addition, plasma separated from the cells may be stored for up to 12 months at ≤-18°C with three freeze/thaw cycles. Refer to Figure 2.

Figure 2 Sample storage conditions for living donor sample



- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

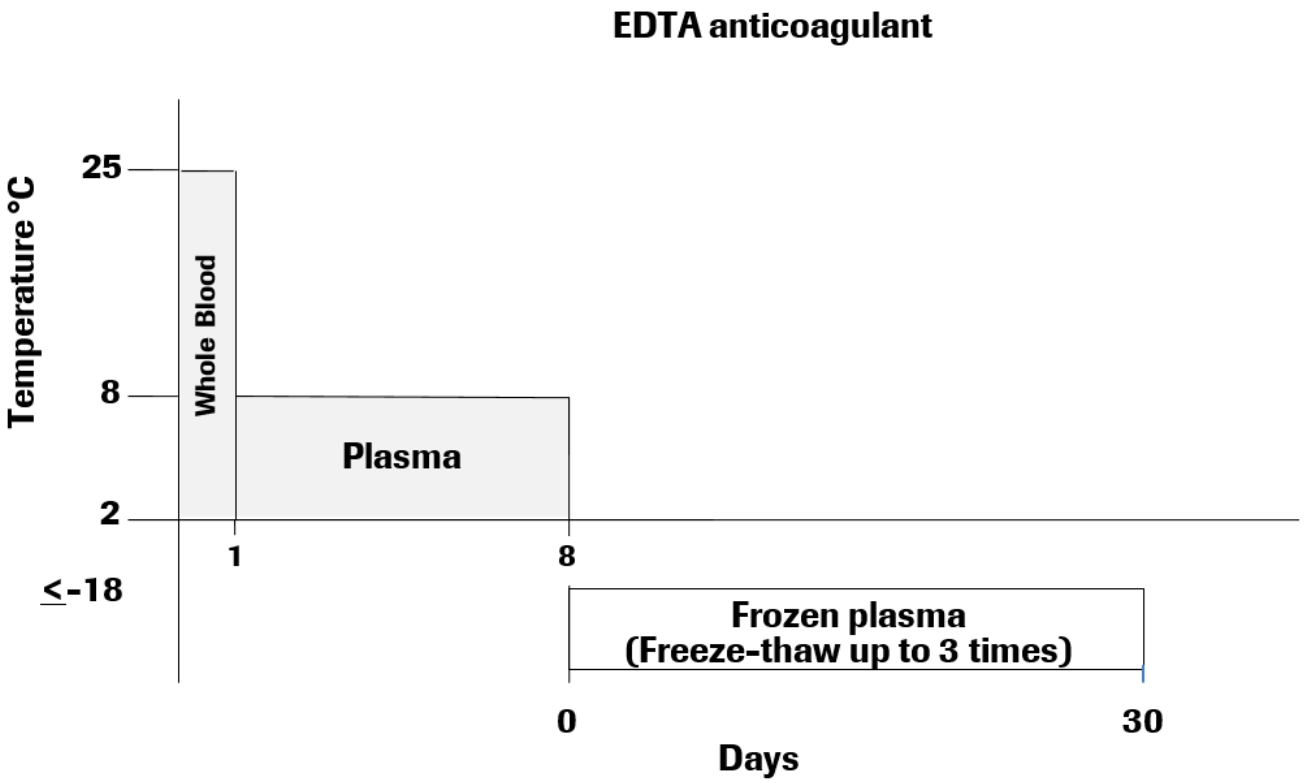
Cadaveric blood samples

Cadaveric blood samples collected in EDTA anticoagulant tubes and/or in serum clot tubes may be used with **cobas**® WNV. Follow the sample collection tube/bag manufacturer instructions for handling and centrifugation.

- Cadaveric blood collected in EDTA anticoagulant may be stored for up to 8 days at 2-8°C with the following conditions:
  - Samples must be centrifuged and plasma must be separated from cells within 24 hours of draw.
  - For storage above 8°C, samples may be stored at up to 25°C, for 24 hours.

Other than noted above, cadaveric EDTA plasma separated from the cells may be stored for up to 30 days at ≤-18°C with up to three freeze/thaw cycles. Refer to Figure 3.

Figure 3 Sample storage conditions for cadaveric sample



- Cadaveric blood collected in serum clot tubes may be stored for up to 3 days at 2-8°C with the following conditions:
  - Serum samples must be centrifuged within 24 hours of draw.
  - For storage above 8°C, samples may be stored at up to 25°C, for 4 hours.

- If living donor and/or cadaveric samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

Instructions for use

Automated sample pipetting and pooling (optional)

Either the **cobas p** 680 instrument, or **cobas® Synergy** Core can be used as an optional component of the **cobas®** 6800/8800 Systems used for automated pipetting and pooling of aliquots of multiple primary samples into one pooled sample. Refer to the **cobas p** 680 instrument Operator's Manual or to the **cobas® Synergy** software User Assistance for more information.

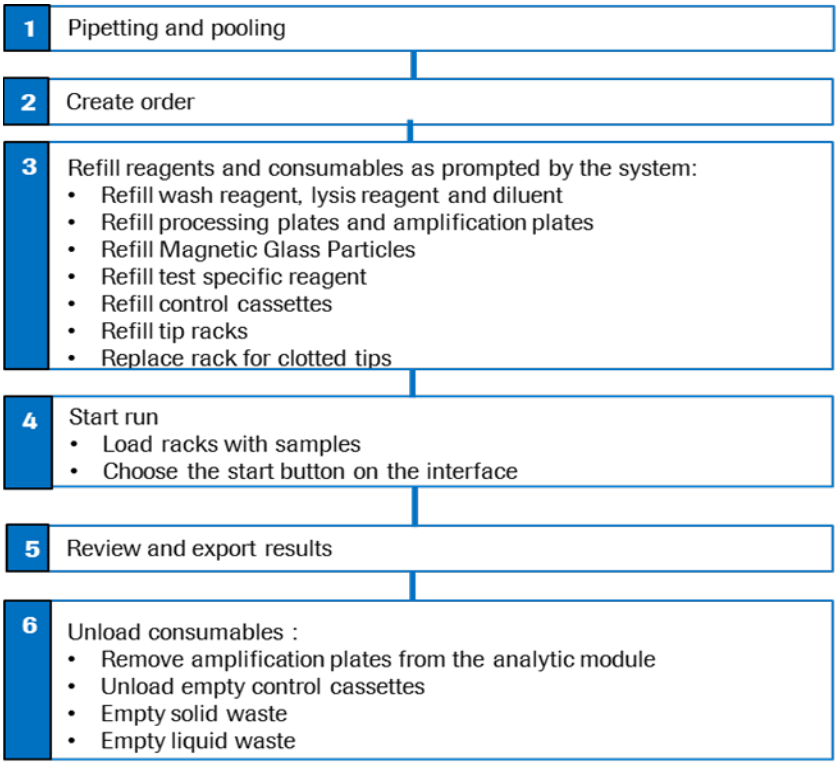
Procedural notes

- Do not use **cobas®** WNV reagents, **cobas®** WNV Control Kit, **cobas®** NHP Negative Control Kit, or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the **cobas®** 6800/8800 Systems Operator's Manual or to the **cobas® Synergy** software User Assistance as applicable for details on optional pooling procedures for proper maintenance of instruments.

Running the cobas® WNV

The test procedure is described in detail in the **cobas®** 6800/8800 Systems Operator's Manual and the **cobas p** 680 instrument Operator's Manual or to the **cobas® Synergy** software User Assistance as applicable for details on optional pooling procedures. Figure 4 below summarizes the procedure.

Figure 4 **cobas®** WNV procedure



Results

The **cobas**® 6800/8800 Systems Software automatically detects WNV RNA simultaneously for the samples and controls.

Quality control and validity of results

- One negative control [(–) C] and one positive control [WNV (+) C] are processed with each batch.
- In the **cobas**® 6800/8800 software and/or report, check for flags and their associated results to ensure the batch validity.
- The batch is valid if no flags appear for both controls.

Invalidation of results is performed automatically by the **cobas**® 6800/8800 software based on negative and positive control failures.

Control flags

Table 9 Control flags for negative and positive controls

Negative Control	**Flag**	**Result**	**Interpretation**
(–) C	Q02	Invalid	The entire batch is assigned invalid if the result for the (-) C is invalid.
Positive Control	**Flag**	**Result**	**Interpretation**
WNV (+) C	Q02	Invalid	The entire batch is assigned invalid if the result for the WNV (+) C is invalid.

If the batch is invalid, repeat testing of the entire batch including samples and controls.

Interpretation of results

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid donor sample results dependent on flags obtained for the individual samples.
- Sample results are valid only if the respective positive controls and the negative control of the corresponding batch are valid.

Two parameters are measured simultaneously for each sample: WNV and the internal control. Final sample results for **cobas**® WNV are reported by the software. In addition to the overall results, individual target results will be displayed in the **cobas**® 6800/8800 software and should be interpreted as follows:

Table 10 Target results for individual target result interpretation

Target results	**Interpretation**
WNV Non-Reactive	No target signal detected for WNV and IC signal detected.
WNV Reactive	Target signal detected for WNV and IC signal may be or may not be detected.
Invalid	Target and internal control signal not detected.

Procedural limitations

- **cobas®** WNV has been evaluated only for use in combination with the **cobas®** WNV Control Kit, **cobas®** NHP Negative Control Kit, **cobas omni** MGP Reagent, **cobas omni** Lysis Reagent, **cobas omni** Specimen Diluent, and **cobas omni** Wash Reagent for use on the **cobas®** 6800/8800 Systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- Do not use heparinized plasma with this test because heparin has been shown to inhibit PCR.
- Detection of WNV RNA is dependent on the number of virus particles present in the sample and may be affected by sample collection, storage and handling, patient factors (i.e., age, presence of symptoms), and/or stage of infection and pool size.
- Though rare, mutations within the highly conserved regions of a viral genome covered by **cobas®** WNV, may affect primers and/or probe binding resulting in the failure to detect presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures for method correlation.
- Other members of the JEV serocomplex (St. Louis encephalitis, Japanese encephalitis, Murray Valley encephalitis, Kunjin virus, and Usutu virus) may also be reactive with **cobas®** WNV.

Non-clinical performance evaluation

Key performance characteristics – Living donor samples

Limit of Detection (LoD)

Roche secondary standard/virus isolate

The LoDs of **cobas**® WNV for WNV lineage 1 and 2 RNA were determined using the following standard:

- Roche Secondary Standard for WNV lineage 1, calibrated against the Health Canada WNV Reference Standard (Infectious Diseases, Canadian Blood Services, 1800 Alta Vista, Ottawa, Ontario, K1G 4J5)
- WNV lineage 2 isolate ISS0513 provided by the National Centre for Immunobiologicals Research and Evaluation, Istituto Superiore di Sanità (ISS), Rome, Italy[41]

For the Roche Secondary Standard, three independent dilution series of WNV lineage 1 were prepared with normal, virus-negative (WNV) human EDTA-plasma. Each dilution series was tested using three different lots of **cobas**® WNV kits with 21 replicates per lot, for a total of 189 replicates per concentration.

For the WNV lineage 2 isolate, panels were prepared by dilution of stock material into normal, virus-negative (WNV) human EDTA-plasma. Each dilution series was tested using three different lots of **cobas**® WNV kits with approximately eight replicates per lot, for a total of approximately 72 replicates per concentration.

For WNV lineage 1 and 2 viruses, PROBIT analysis on the data combined across dilution series and reagent lots was used to estimate the LoD, along with the lower and upper limit of the 95% confidence interval (Table 11). The reactivity rates observed in the LoD studies for lineage 1 and 2 are summarized in Table 12 and Table 13, respectively.

Table 11 Results of PROBIT analysis on LoD data collected with viral standard in EDTA plasma

Analyte	Measuring units	LoD	Lower 95% confidence limit	Upper 95% confidence limit
WNV Lineage 1	copies/mL	12.9	10.8	16.3
WNV Lineage 2	copies/mL	6.2	4.8	8.9

Table 12 Reactivity rates summary for WNV Lineage 1 in EDTA plasma

WNV RNA concentration (copies/mL)	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
18.0	187	188	99.5%	97.5%
9.0	173	188	92.0%	88.0%
4.5	139	188	73.9%	68.1%
2.7	93	189	49.2%	43.0%
0.9	53	189	28.0%	22.7%

Table 13 Reactivity rates summary for WNV Lineage 2 in EDTA plasma

WNV RNA concentration (copies/mL)	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
22.8	72	72	100.0%	95.9%
15.2	72	72	100.0%	95.9%
7.6	69	72	95.8%	89.6%
3.8	64	72	88.9%	80.8%
2.3	53	72	73.6%	63.7%
0.8	30	72	41.7%	31.8%

Inclusivity

The performance of **cobas**® WNV to detect flavivirus variants of WNV was determined by testing unique cultured isolates for each variant. A total of 10 individual WNV Lineage 1 positive cultured isolates were tested after dilution with normal, virus-negative (WNV) human EDTA-plasma at the concentration of approximately 36 copies/mL. All 10 cultured samples were detected (Table 14).

For the flavivirus variants of WNV, a total of two positive cultured isolates of Japanese encephalitis virus (JEV) were tested with four replicates after dilution with normal, virus-negative (WNV) human EDTA-plasma. A total of one positive cultured isolate of Saint Louis encephalitis virus (SLEV), Murray Valley encephalitis virus (MVEV) and Kunjin virus (KUNV) was tested using four replicates of each isolate after log dilutions were prepared with normal, virus-negative (WNV) citrate plasma. All cultured isolates were detected (Table 15).

Table 14 Cultured isolates of WNV Lineage 1

Flavivirus variants	Concentration (Copies/mL)	% Reactive (reactive/samples tested)
WNV 1	36	100.0% (10/10)

Table 15 Cultured isolates of WNV flavivirus variants

Sample Dilution	% Reactive (reactive/valid replicates tested) JEV	% Reactive (reactive/valid replicates tested) SLEV	% Reactive (reactive/valid replicates tested) MVEV	% Reactive (reactive/valid replicates tested) KNUV
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)

Analytical specificity

The analytical specificity of **cobas**® WNV was evaluated for cross-reactivity with 27 microorganisms at 10^6 particles, copies, or PFU/mL, which included 20 viral isolates, six bacterial strains and one yeast isolate (Table 16). The microorganisms were added to normal, virus-negative (WNV) human EDTA-plasma and tested without WNV and with WNV added to a concentration of approximately 3 x LoD of **cobas**® WNV. The tested microorganisms do not cross-react or interfere with **cobas**® WNV.

Table 16 Microorganisms tested for analytical specificity

Viruses	**Bacteria**	**Yeast**
Adenovirus 5	*Escherichia coli*	*Candida albicans*
Chikungunya Virus	*Propionibacterium acnes*	-
Cytomegalovirus	*Staphylococcus aureus*	-
Dengue Virus type 1	*Staphylococcus epidermidis*	-
Epstein-Barr Virus	*Streptococcus viridans*	-
Herpes Simplex Virus type 1	*Staphylococcus haemolyticus*	-
Herpes Simplex Virus type 2	-	-
Hepatitis A Virus	-	-
Hepatitis B Virus	-	-
Hepatitis C Virus	-	-
Hepatitis E Virus	-	-
Hepatitis G Virus Human Immunodeficiency Virus (HIV-1 Group M) Human Immunodeficiency Virus (HIV-2)	-	-
Human T-cell Lymphotropic Virus type I	-	-
Human T-cell Lymphotropic Virus type II	-	-
Human Herpes Virus 6 B Influenza Virus A	-	-
Usutu Virus*	-	-
Varicella Zoster Virus	-	-

* Usutu virus showed cross reactivity when tested without added WNV due to nucleotide sequence homology with WNV.
The **cobas**® WNV test may cross-react with clinically relevant viruses belonging to the family of Flaviviridae.

Plasma samples from each of the disease states (Table 17) were tested without WNV and with WNV added to a concentration of approximately 3 x LoD of **cobas**® WNV. These disease states do not cross-react or interfere with **cobas**® WNV.

Table 17 Analytical specificity - known virus-positive samples tested for cross reactivity

Virus	Virus	Virus
Adenovirus type 5	Hepatitis A Virus	Human T-cell Lymphotropic Virus type II
Cytomegalovirus	Hepatitis B Virus	Herpes Simplex Virus type1
Dengue Virus	Hepatitis C Virus	Herpes Simplex Virus type 2
Epstein-Barr Virus	Human T-cell Lymphotropic Virus type I	Human Immunodeficiency Virus (HIV-1)

Analytical specificity – interfering substances

Endogenous interference substances

Plasma samples with abnormally high levels of triglycerides (up to 35.3 g/L), hemoglobin (up to 4.7 g/L), unconjugated bilirubin (up to 0.21 g/L), albumin (up to 61.3 g/L), and human DNA (up to 0.004g/L) were tested without WNV and with WNV added to a concentration of approximately 3 x LoD of **cobas**® WNV. Samples containing these endogenous substances did not interfere with the sensitivity or specificity of **cobas**® WNV.

Exogenous interference substances

Normal, virus-negative (WNV) human EDTA-plasma samples containing abnormally high concentrations of drugs (Table 18) were tested without WNV and with WNV added to a concentration of 3 x LoD of **cobas**® WNV. These exogenous substances did not interfere with the sensitivity or specificity of **cobas**® WNV.

Table 18 Clinical samples tested with drugs

Name of drug tested	Concentration
Acetaminophen	1324 µmol/L
Acetylsalicylic Acid	3620 µmol /L
Ascorbic Acid	342 µmol/L
Atorvastatin	600 µg Eq/L
Fluoxetine	11.2 µmol/L
Ibuprofen	2425 µmol/L
Loratadine	0.78 µmol/L
Nadolol	3.88 µmol/L
Naproxen	2170 µmol/L
Paroxetine	3.04 µmol/L
Phenylephrine HCL	491 µmol/L
Sertraline	1.96 µmol/L

Whole system failure

The whole system failure rate for **cobas**® WNV was determined by testing 100 replicates of EDTA plasma spiked with WNV. These samples were tested at a target concentration of approximately 3 x LoD and were run in pools of one (undiluted). The study was performed using the **cobas**® 8800 System with **cobas p** 680 instrument (pipetting and pooling).

The results of this study determined that all replicates were reactive for WNV, resulting in a whole system failure rate of 0%. The two-sided 95% exact confidence interval was 0% for the lower bound and 3.62% for the upper bound [0%: 3.62%].

FDA/CBER panel evaluation

The sensitivity of **cobas**® WNV was determined by testing the FDA/CBER WNV Lot Release panel (Table 19).

In total 10 panel members were tested undiluted in each three replicates across three reagent lots (one replicate per panel member per reagent lot).

The results of this study determined that all FDA/CBER panel members with WNV titers of 5 cp/mL to 100 cp/mL were detected by **cobas**® WNV and that all panel members of 0 cp/mL were non-reactive for WNV, irrespective of the reagent lot.

Table 19 Summary FDA/CBER WNV lot release panel results

Panel Member	Concentration (cp/mL)	% Reactive (reactive/replicates tested)
#5	0	0% (0/3)
#3	0	0% (0/3)
#10	5	100% (3/3)
#9	5	100% (3/3)
#2	10	100% (3/3)
#12	10	100% (3/3)
#13	50	100% (3/3)
#4	50	100% (3/3)
#1	100	100% (3/3)
#7	100	100% (3/3)

Key performance characteristics – Cadaveric samples

Analytical sensitivity

The analytical sensitivity of the **cobas**® WNV test for WNV lineage I in cadaveric EDTA samples was assessed using a Roche Secondary Standard (traceable to the Health Canada WNV Reference Standard (Infectious Diseases, Canadian Blood Services, 1800 Alta Vista, Ottawa, K1G4J5).

A total of two (2) independent dilution series of 6 concentrations and a blank were prepared by diluting the WNV secondary standard in negative pooled moderately hemolyzed cadaveric EDTA samples and pooled highly hemolyzed cadaveric EDTA plasma samples. Each dilution series was tested using one of two unique reagent lots of the **cobas**® WNV test.

The results are summarized in Table 20 and Table 21.

Table 20 Analytical sensitivity summary for WNV lineage 1 in moderately-hemolyzed cadaveric samples

WNV concentration (cp/mL)	Number of Reactives	Number of Valid Replicates	% Reactive	95% lower confidence bound (one-sided)
240	42	42	100%	93.1%
120	42	42	100%	93.1%
90	42	42	100%	93.1%
60	41	42	98%	89.2%
30	29	42	69%	55.4%
15	18	42	43%	29.8%
0	0	41	0%	0.0%

Table 21 Analytical sensitivity summary for WNV lineage 1 in highly-hemolyzed cadaveric samples

WNV concentration (cp/mL)	Number of Reactives	Number of Valid Replicates	% Reactive	95% lower confidence bound (one-sided)
240	42	42	100%	93.1%
120	42	42	100%	93.1%
90	40	42	95%	85.8%
60	39	42	93%	82.6%
30	28	42	67%	52.9%
15	21	41	51%	37.4%
0	0	42	0%	0.0%

Sensitivity using clinical samples

The clinical sensitivity of **cobas**® WNV for WNV RNA was evaluated by testing a total of 60 individual virus-negative cadaveric samples, of those 35 individual samples were classified as moderately hemolyzed (straw to pink colored) and 25 individual samples were classified as highly hemolyzed (red to brown colored). In addition a total of 60 individual virus-negative living donor samples were tested. All cadaveric and living donor samples were divided evenly across three reagent lots, five clinical samples spiking groups (for WNV) with 12 samples per group. Each cadaveric and living donor sample was spiked with a unique clinical samples (WNV) at approximately 5 x LoD of the respective sample. Each cadaveric sample was diluted 1:5.6 with **cobas omni** Specimen Diluent on the instrument and tested using the cadaveric sample testing procedure.

All of the cadaveric and the living-donor samples had a reactive rate of 100% (95% confidence interval: 94.0 - 100%). The clinical sensitivity observed in cadaveric sample was equivalent to the sensitivity observed in living donor samples as determined by Fisher's Exact Test and summarized in Table 22.

Table 22 Summary of reactivity rate in cadaveric and living donor samples in EDTA plasma

Analyte	Cadaveric sample % Reactive (Number of reactive /Number of samples tested)	Living donor sample % Reactive (Number of reactive/Number of samples tested)
WNV	100% (60/60)	100% (60/60)
Fisher's Exact Test, p-value (α= 0.05)	No significant differences in reactive rates (p = 1.000)	No significant differences in reactive rates (p = 1.000)

Specificity

The specificity of **cobas**® WNV in cadaveric EDTA plasma and serum samples was evaluated and compared with the specificity in living donor samples by testing single replicates of 64 individual cadaveric EDTA plasma samples, 62 individual cadaveric serum samples, 60 individual seronegative living donor plasma samples and 60 individual living donor serum samples. Of the 64 cadaveric EDTA plasma samples, 40 individual donor samples were classified as moderately hemolyzed (straw to pink colored) and 24 individual samples were classified as highly hemolyzed (red to brown colored). Of the 62 cadaveric serum samples, 42 individual samples were classified as moderately hemolyzed and 20 individual samples were classified as highly hemolyzed.

The studies were performed with three independent **cobas**® WNV reagent lots. Each cadaveric sample was diluted 1:5.6 with **cobas omni** Specimen Diluent on the instrument and tested using the cadaveric sample testing procedure. All the cadaveric and living donor EDTA plasma and serum samples were non-reactive for 100% specificity. The specificity observed for cadaveric samples was equal to the specificity observed for living-donor samples as determined by the Fisher's Exact Test (α = 0.05) as summarized in Table 23.

Table 23 Summary of specificity in cadaveric and living-donor samples in EDTA plasma and serum

Matrices	Sample type	Number of non-reactive	Number of samples tested	% Non-reactive	Two-sided 95% Confidence Interval
EDTA plasma	Cadaveric donor	64	64	100%	94.4% - 100%
EDTA plasma	Living donor	60	60	100%	94.0% - 100%
Serum	Cadaveric donor	62	62	100%	94.2% - 100%
Serum	Living donor	60	60	100%	94.0% - 100%
-	Overall results using Fisher's Exact Test (α= 0.05)	Specificity for cadaveric sample and living-donor samples are equivalent: Fisher's Exact Test, p = 1.000	Specificity for cadaveric sample and living-donor samples are equivalent: Fisher's Exact Test, p = 1.000	Specificity for cadaveric sample and living-donor samples are equivalent: Fisher's Exact Test, p = 1.000	Specificity for cadaveric sample and living-donor samples are equivalent: Fisher's Exact Test, p = 1.000

Reproducibility

The reproducibility of **cobas**® WNV on the **cobas**® 6800/8800 Systems was determined using 20 cadaveric EDTA plasma samples (moderately and highly hemolyzed) spiked with Roche Secondary Standard for WNV RNA to approximately 5 x LoD of **cobas**® WNV. The results were compared to the reproducibility obtained with 20 living-donor EDTA plasma samples spiked with the Roche Secondary Standard to approximately 5 x LoD of **cobas**® WNV.

Testing was performed for the following variable components:

- day-to-day variability over 6 days
- lot-to-lot variability using three different reagent lots of **cobas**® WNV

One replicate was tested with each of the three reagent lots over six days for up to 18 replicates per cadaveric and living donor sample. Each cadaveric sample was diluted 1:5.6 with **cobas omni** Specimen Diluent on the instrument and tested using the cadaveric sample testing procedure. All valid reproducibility data were evaluated by comparing the reactive rates of living donors and cadaveric samples (two-sided 95% Confidence Intervals) across all variable components. The Fisher's exact p value was calculated for the test of statistical significance of the difference between proportions of reactives observed with cadaveric and living donor samples. No significant differences were observed.

cobas® WNV is reproducible over multiple days and reagent lots for cadaveric and living donor EDTA plasma samples. The results from reagent lot-to-lot variability are summarized in Table 24.

Table 24 cobas® WNV reagent lot-to-lot reproducibility summary for cadaveric and living-donor samples

Analyte	Reagent lot	Sample type	% Reactive (reactive/valid replicates)	Lower limit of 95% Confidence interval	Upper limit of 95% Confidence Interval	Significant difference using Fisher's Exact Test (α = 0.05)
WNV	1	Cadaveric	100.0% (120/120)	97.0%	100.0%	p-value = 1.0000
WNV	1	Living donor	100.0% (120/120)	97.0%	100.0%	p-value = 1.0000
WNV	2	Cadaveric	100.0% (120/120)	97.0%	100.0%	p-value = 1.0000
WNV	2	Living donor	100.0% (120/120)	97.0%	100.0%	p-value = 1.0000
WNV	3	Cadaveric	100.0% (120/120)	97.0%	100.0%	p-value = 1.0000
WNV	3	Living donor	100.0% (120/120)	97.0%	100.0%	p-value = 1.0000
WNV	3	Living donor	100.0% (120/120)	97.0%	100.0%	p-value = 1.0000

Matrix equivalency

The matrix equivalency of the **cobas**® WNV test on the **cobas**® 6800/8800 Systems was evaluated between cadaveric EDTA plasma and cadaveric serum specimens to determine whether there is a sample matrix effect on the sensitivity and reproducibility of the test. The study was conducted using 20 pairs of cadaveric samples, with each set consisting of one cadaveric EDTA plasma sample and one cadaveric serum sample from a single donor. Of those, fifteen donor sets were moderately-hemolyzed (straw to pink colored), and five of the donor sets were highly-hemolyzed (red to brown colored). Each pair of cadaveric serum and plasma samples were spiked with approximately 3 x LoD of WNV Secondary Standard before testing (10 replicates per specimen) with the **cobas**® WNV Test.

The observed **cobas**® WNV test reactive rates are statistically equivalent in either cadaveric EDTA plasma or cadaveric serum both in highly-hemolyzed and moderately-hemolyzed samples, The results from cadaveric matrix equivalency are summarized in Table 25.

Table 25 cobas® WNV test Matrix Equivalency for cadaveric samples

Analyte	Hemolysis Level	Sample type	Total number tested	Total number reactive	Reactive Rate	Lower limit of 95% Confidence interval	Upper limit of 95% Confidence Interval	p-value
WNV	Moderately Hemolyzed	Plasma	150	149	99.3%	96.3	100.0	1.0000
WNV	Moderately Hemolyzed	Serum	150	149	99.3%	96.3	100.0	1.0000
WNV	Highly Hemolyzed	Plasma	50	50	100.0%	92.9	100.0	0.5283
WNV	Highly Hemolyzed	Serum	50	49	98.0%	89.4	100.0	0.5283

Clinical performance evaluation

Clinical sensitivity – testing of known West Nile virus positive samples

The clinical sensitivity of **cobas**® WNV was evaluated using 530 individual clinical samples that were known to be positive based on NAT testing. The study was conducted at four testing laboratories, with each site testing approximately 135 samples, both neat and diluted 1:6, using three different lots of **cobas**® WNV. No valid result was obtained for two of the neat samples, so only 528 neat results were included in analysis. All 530 diluted samples produced valid results.

The sensitivity of **cobas**® WNV with neat samples in this study was 99.1% (95% Confidence Interval: (CI): 97.8% to 99.7%) and with diluted (1:6) samples was 96.2% (95% CI: 94.2% to 97.7%) (Table 26). The five non-reactive neat samples had low viral titers. The twenty non-reactive diluted samples were derived from neat samples with low viral titers.

Table 26 Clinical sensitivity of known West Nile virus positive samples

-	Number of Samples Tested	Number of Samples Reactive	Number of Samples Non-Reactive	Sensitivity (%)	Sensitivity (95% CI**) Lower Limit	Sensitivity (95% CI**) Upper Limit
Neat	528*	523	5	99.1	97.8	99.7
1:6	530	510	20	96.2	94.2	97.7

*No valid result was obtained for 2 of the neat samples.

**Clopper-Pearson Exact method

Clinical specificity

The clinical specificity of **cobas**® WNV was evaluated by testing randomly-selected blood donations at four external laboratory sites. Individual samples and samples in pools of six were tested. Three different **cobas**® WNV reagent lots were used in the study. Clinical specificity of the **cobas**® WNV was calculated as the percentage (95% two-sided CI) of WNV donor status-negative donors who had **cobas**® WNV non-reactive results. There were 63,243 evaluable donations from pooled testing and 10,823 evaluable donations from individual testing.

Pooled testing results

Table 27 shows the calculation of the clinical specificity of **cobas**® WNV for the 63,243 evaluable donors from pooled testing. The clinical specificity of the **cobas**® WNV from pooled testing was 100% (63,243/63,243; 95% CI: 99.99% to 100%) in this study.

Table 27 Clinical specificity of **cobas**® WNV – pooled testing

cobas® **WNV Result**	**WNV Donation Status* Positive**	**WNV Donation Status* Negative**	**Total**
WNV Reactive	0	0	0
WNV Non-Reactive	0	63,243	63,243
Total	0	63,243	63,243
Clinical Specificity (95% CI)**	-	**100% (99.99%, 100%)**	-

* WNV Donor Status was assigned based on test reactivity patterns on the index donation and, if present, follow-up donation(s).

**Clopper-Pearson Exact method

The **cobas**® WNV pool specificity for index donations was 100% (10,573/10,573; 95% CI: 99.96% to 100%). None of the 10,573 pools of six were **cobas**® WNV reactive. An invalid rate of 1.6% due to internal control or instrument failures was observed for pooled sample results.

Individual testing results

Table 28 shows the calculation of the clinical specificity of **cobas**® WNV for the 10,823 evaluable donors from individual testing. The clinical specificity of **cobas**® WNV from individual testing was 100% (10,823/10,823; 95% CI: 99.96% to 100%) in this study. An invalid rate of 0.3% due to internal control, instrument failures, protocol deviations, or other incidents was observed for individual sample results.

Table 28 Clinical specificity of **cobas**® WNV – individual testing

cobas® **WNV Result**	**WNV Donation Status* Positive**	**WNV Donation Status* Negative**	**Total**
WNV Reactive	0	0	0
WNV Non-Reactive	0	10,823	10,823
Total	0	10,823	10,823
Clinical Specificity (95% CI)**	-	**100% (99.96%, 100%)**	-

* WNV Donor Status was assigned based on test reactivity patterns on the index donation and, if present, follow-up donation(s).

** Clopper-Pearson Exact method

Reproducibility

The reproducibility of **cobas**® WNV for use on the **cobas**® 6800/8800 Systems was established by testing an eight member panel composed of two negative plasma samples and two samples positive for WNV at three different concentrations (approximately 0.5 x, 1.0 x, and 3.0 x the LoD of **cobas**® WNV).

Operators at each of three sites with the **cobas**® 8800 performed five days of testing with each of three lots of **cobas**® WNV reagents and two valid panel runs (i.e., two batches, each batch composed of one panel and two independent controls) per day were completed to yield up to 180 tests per panel member virus type at each of the three concentrations.

All valid batches and test results were analyzed by calculating the percentage of reactive test results for each panel member (Table 29). This study demonstrated that **cobas**® WNV for use on the **cobas**® 6800/8800 Systems shows reproducible performance across the variables assessed (lot, site/instrument, day, batch, and within batch) for detecting WNV.

Table 29 Test results summarized by site, lot, day, and batch (positive panel members)

Viral Concentration	Site ID	Site % Positive Results	Lot ID	Lot % Positive Results	Day ID	Day % Positive Results	Batch ID	Batch % Positive Results
0.5 x LoD	1	86.7% (52/60)	1	85.0% (51/60)	1	94.4% (34/36)	1	81.1% (73/90)
0.5 x LoD	2	90.0% (54/60)	2	91.7% (55/60)	2	72.2% (26/36)	2	91.1% (82/90)
0.5 x LoD	3	81.7% (49/60)	3	81.7% (49/60)	3	88.9% (32/36)	-	-
0.5 x LoD	-	-	-	-	4	86.1% (31/36)	-	-
0.5 x LoD	-	-	-	-	5	88.9% (32/36)	-	-
1.0 x LoD	1	95.0% (57/60)	1	96.7% (58/60)	1	86.1% (31/36)	1	93.3% (83/89)
1.0 x LoD	2	100% (59/59)	2	88.3% (53/60)	2	94.4% (34/36)	2	92.2% (83/90)
1.0 x LoD	3	83.3% (50/60)	3	93.2% (55/59)	3	94.3% (33/35)	-	-
1.0 x LoD	-	-	-	-	4	94.4% (34/36)	-	-
1.0 x LoD	-	-	-	-	5	94.4% (34/36)	-	-
3.0 x LoD	1	98.3% (59/60)	1	98.3% (59/60)	1	100% (36/36)	1	98.9% (89/90)
3.0 x LoD	2	100% (60/60)	2	100% (60/60)	2	100% (36/36)	2	100% (90/90)
3.0 x LoD	3	100% (60/60)	3	100% (60/60)	3	97.2% (35/36)	-	-
3.0 x LoD	-	-	-	-	4	100% (36/36)	-	-
3.0 x LoD	-	-	-	-	5	100% (36/36)	-	-

Note: Within this table, separate summaries of the results from all replicates for each panel member virus concentration are presented for the variables site, lot, day and batch.

*Clopper-Pearson Exact method

Additional information

Key test features

Sample type	Plasma
Minimum amount of sample required for living donor	1000 µL
Amount of sample processed for living donor	850 µL
Minimum amount of sample required for cadaveric donor	300 µL
Amount of sample processed for cadaveric donor	150 µL
Test duration	Results are available within less than 3.5 hours after loading the sample on the system.

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 30 Symbols used in labeling for Roche PCR diagnostics products

	Ancillary Software		*In vitro* diagnostic medical device
	Authorized representative in the European Community		Lower Limit of Assigned Range
	Barcode Data Sheet		Manufacturer
	Batch code		Store in the dark
	Biological risks		Contains sufficient for <*n*> tests
	Catalogue number		Temperature limit
	Consult instructions for use		Test Definition File
	Contents of kit		Upper Limit of Assigned Range
	Distributed by		Use-by date
	For IVD performance evaluation only		Global Trade Item Number
Rx Only	US Only: Federal law restricts this device to sale by or on the order of a physician.		Date of manufacture
	CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device		

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 31 Manufacturer and distributors

Manufactured in the United States

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com

U.S. License No. 1636

Made in USA

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA
(For Technical Assistance call the
Roche Response Center
toll-free: 1-800-526-1247)

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Trademarks and patents

This product is covered by one or more of US Patent Nos. 8962293, 9102924, 8609340, 9234250, 8097717, 8192958, 10059993, 10358675, and foreign equivalent patents of each.

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The trademark "Armored RNA®" is owned by Asuragen, Inc. and Cenetron Diagnostics, Ltd.

All other product names and trademarks are the property of their respective owners.

Carryover prevention technology in the AmpErase enzyme is covered by U.S. Patent 7,687,247 owned by Life Technologies and licensed to Roche Molecular Systems, Inc.

See http://www.roche-diagnostics.us/patents

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Document revision

Document Revision Information	
Doc Rev. 6.0 02/2020	Addition of cadaveric serum sample claims. Updated the harmonized symbol page, distributors addresses, and trademarks and patents section. Please contact your local Roche Representative if you have any questions.
07/2020	Updated the IFU formatting for Section 508 compliance. No incrementation of SAP or doc revision numbers. Updated harmonized symbol page. Please contact your local Roche Representative if you have any questions.
Doc Rev. 7.0 03/2021	Updated hazard warnings. Added Made in statement. Updated **Trademarks and patents** section. Please contact your local Roche Representative if you have any questions.