cobas® WNV

For in vitro diagnostic use

cobas® WNV – 192	P/N: 09171142190
cobas® WNV – 480	P/N: 09040927190
cobas® WNV Control Kit	P/N: 09040935190
cobas® NHP Negative Control Kit	P/N: 09051554190
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

The **cobas**® WNV test for use on **cobas**® 5800/6800/8800 systems is a qualitative in vitro 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 and for testing of cadaveric (non-heart beating) donors.

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

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 may be tested in pools comprised of aliquots of individual samples. For donations from cadaveric (non-heart beating) organ and tissue donors, samples may only be screened as individual sample.

This test may also be used as an aid in diagnosis of WNV in samples collected from individuals suspected of infection with WNV by their healthcare provider.

When used as an aid in diagnosis, plasma samples should only be tested individually.

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 3 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] Ninety-four percent 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

The **cobas**® WNV test is a qualitative test that is run on the **cobas**® 5800/6800/8800 systems. The **cobas**® WNV test 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 or an individual sample for aid in diagnosis.

Principles of the procedure

The **cobas**® WNV test 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**® 5800 system consists of a single, integrated instrument. 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**® 5800 or 6800/8800 system software which assigns test results for all tests as non-reactive, reactive, or invalid. When using the **cobas**® 5800/6800/8800 system, 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.

For donor screening, samples can either be tested individually or, optionally, can be tested in pools consisting of multiple samples.

If pooling is to be performed, the **cobas**® p 680 instrument (for **cobas**® 6800/8800 systems), or **cobas**® **Synergy** software with the Hamilton Microlab® STAR/STARlet IVD, may optionally be used in a pre-analytical step.

Nucleic acid from the sample and added armored RNA internal control (IC) (which serves as a full process control from sample preparation through amplification/detection) is simultaneously extracted. The IC monitors for interference that could cause false negative results. Potentially affected samples are invalidated. 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 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.

The **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 test

Store at 2-8°C

192 test cassette (P/N 09171142190)

480 test cassette (P/N 09040927190)

Kit components	Reagent ingredients	Quantity per kit	
		192 tests	**480 tests**
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase, glycerol EUH210: Safety data sheets available on request. EUH208: Contains subtilisin from Bacillus subtilis. May produce an allergic reaction.	22.3 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	21.2 mL	38 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	21.2 mL	38 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	7.5 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	9.7 mL	17.5 mL

Table 2 cobas® WNV Control Kit

Store at 2-8°C

(P/N 09040935190)

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. H412: Harmful to aquatic life with long lasting effects. P261: Avoid breathing mist or vapours. P273: Avoid release to the environment. 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 reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (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 09051554190)

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 mist or vapours. 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 reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (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: Harmful if swallowed. H314: Causes severe skin burns and eye damage. H411: Toxic to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. EUH071: Corrosive to the respiratory tract. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing 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. P391: Collect spillage. 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 8 and Table 9).

** Product safety labeling primarily follows EU GHS guidance

*** Hazardous substance

Reagent storage and handling requirements

Reagents shall be stored and will be handled as specified in Table 5, Table 6 and Table 7.

When reagents are not loaded on the **cobas**® 5800/6800/8800 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 – 192	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

Reagent handling requirements for the cobas® 5800 system

Reagents loaded onto the **cobas**® 5800 system 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**® 5800 system.

Table 6 Reagent expiry conditions enforced by the **cobas**® 5800 system

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 – 192	Date not passed	90 days from first usage	Max 40 runs	Max 36 days**
cobas® WNV – 480	Date not passed	30 days from first usage	Max 40 runs	Max 36 days**
cobas® WNV control Kit	Date not passed	Not applicable*	Not applicable	Max 36 days**
cobas® NHP Negative control Kit	Date not passed	Not applicable*	Not applicable	Max 36 days**
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

* Single use reagents

** Time is measured from the first time that reagent is loaded onto the **cobas**® 5800 system.

Reagent handling requirements for the cobas® 6800/8800 systems

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 7 are met. The system automatically prevents use of expired reagents. Table 7 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 systems.

Table 7 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 – 192	Date not passed	90 days from first usage	Max 40 runs	Max 40 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

* Single use reagents

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

Additional materials required for the cobas® 5800 system

Table 8 Material and consumables for use on **cobas**® 5800 system

Material	P/N
cobas® **omni** Processing Plate 24	08413975001
cobas® **omni** Amplification Plate 24	08499853001
cobas® **omni** Liquid Waste Plate 24	08413983001
CORE Tips with Filter, 1 mL	04639642001
CORE Tips with Filter, 300 µL	07345607001
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 or Solid Waste Bag with Insert	07435967001 or 08030073001

Additional materials required for the cobas® 6800/8800 systems

Table 9 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 Bag with Insert	08030073001

Instrumentation and software required

The **cobas**® WNV analysis package for the **cobas**® 5800 system shall be installed on the **cobas**® 5800 system. The x800 Data Manager software for the **cobas**® 5800 system will be provided with the system. The **cobas**® **Synergy** software shall be installed, if applicable.

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 10 Instrumentation

Equipment	P/N
cobas® 5800 system	08707464001
cobas® 6800 system (Option Moveable)	05524245001 and 06379672001
cobas® 6800 system (Fix)	05524245001 and 06379664001
cobas® 8800 system	05412722001
Sample Supply Module for **cobas**® 6800/8800 systems	06301037001
Options for pipetting and pooling	**P/N**
cobas® **Synergy** software electronic license (for **cobas**® 5800 system only)	09311246001
cobas® p 680 instrument	06570577001
cobas® **Synergy** software electronic license (**cobas**® 6800/8800 systems) (Optional)	09311238001
Hamilton MICROLAB® STAR IVD	04640535001
Hamilton MICROLAB® STARlet IVD	04872649001

Refer to the **cobas**® 5800 system User Assistance or the **cobas**® 6800/8800 systems User Assistance for additional information. Refer to the **cobas**® p 680 instrument User Assistance, 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 infectious materials and the use of the **cobas**® WNV test, the **cobas**® 5800/6800/8800 systems, and optionally the **cobas**® p 680 instrument (for **cobas**® 6800/8800 systems) or the Hamilton MICROLAB® STAR/STARlet IVD with **cobas**® **Synergy** software 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 0.6% sodium hypochlorite in distilled or deionized water 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 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.
- Disruption of the cell-plasma interface or diffusion of material post-centrifugation may result in higher invalid rates.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.
- Inform your local competent authority and manufacturer about any serious incidents which may occur when using this assay.

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 test 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 test kits and **cobas**® **omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- 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 0.6% sodium hypochlorite in distilled or deionized water. 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 User Assistance to properly clean and decontaminate the surface of instrument(s).
- If spills occur on the **cobas**® 5800 instrument, follow the instructions in the **cobas**® 5800 system User Assistance to properly clean and decontaminate the surfaces of the instrument(s).

Sample collection, transport, storage, and pooling

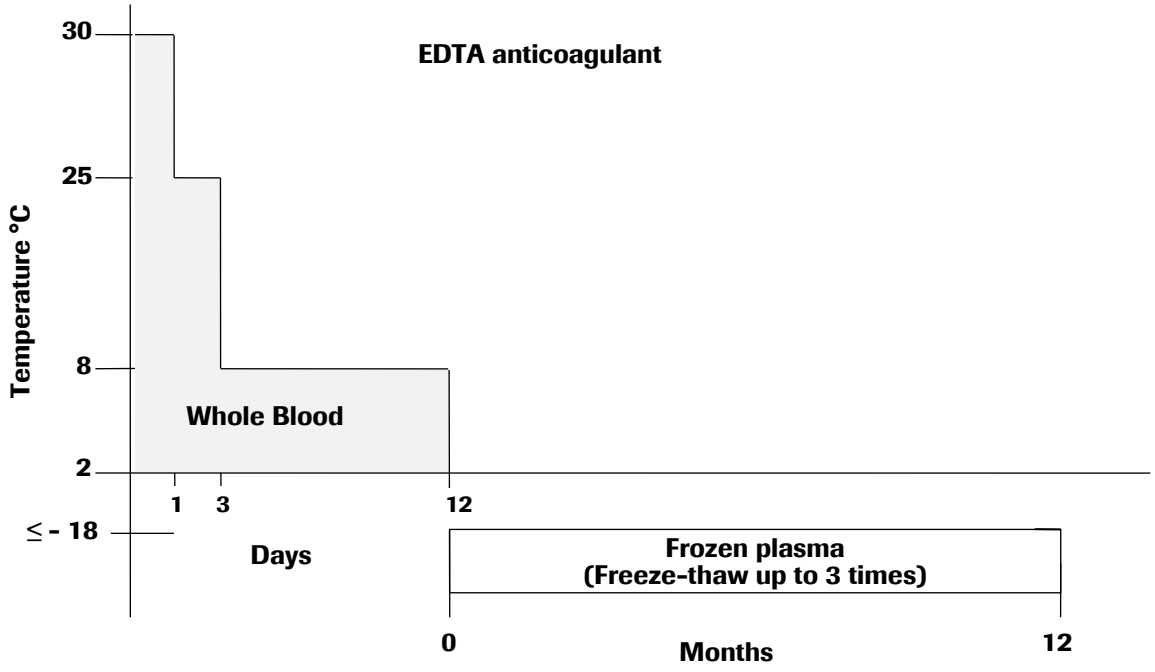
Note: Handle all samples and controls as if they are capable of transmitting infectious agents.
Store all donor samples at specified temperatures.
Sample stability is affected by elevated temperatures.

Living donor and diagnostic samples

- Plasma collected in EDTA, CPD, CPDA1, CP2D anticoagulant may be used with the **cobas**® WNV test. Follow the sample collection tube/bag manufacturer instructions for handling and centrifugation.
- Blood collected in EDTA anticoagulant, Becton-Dickinson EDTA Plasma Preparation Tubes (BD PPT™) or Greiner Vacuette® K2EDTA Plasma Gel Tubes may undergo additional centrifugation at 600 x g for 5 minutes prior to loading, optional pooling or retesting.
- 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, 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 12 months at ≤-18°C with three freeze/thaw cycles. Refer to Figure 1.

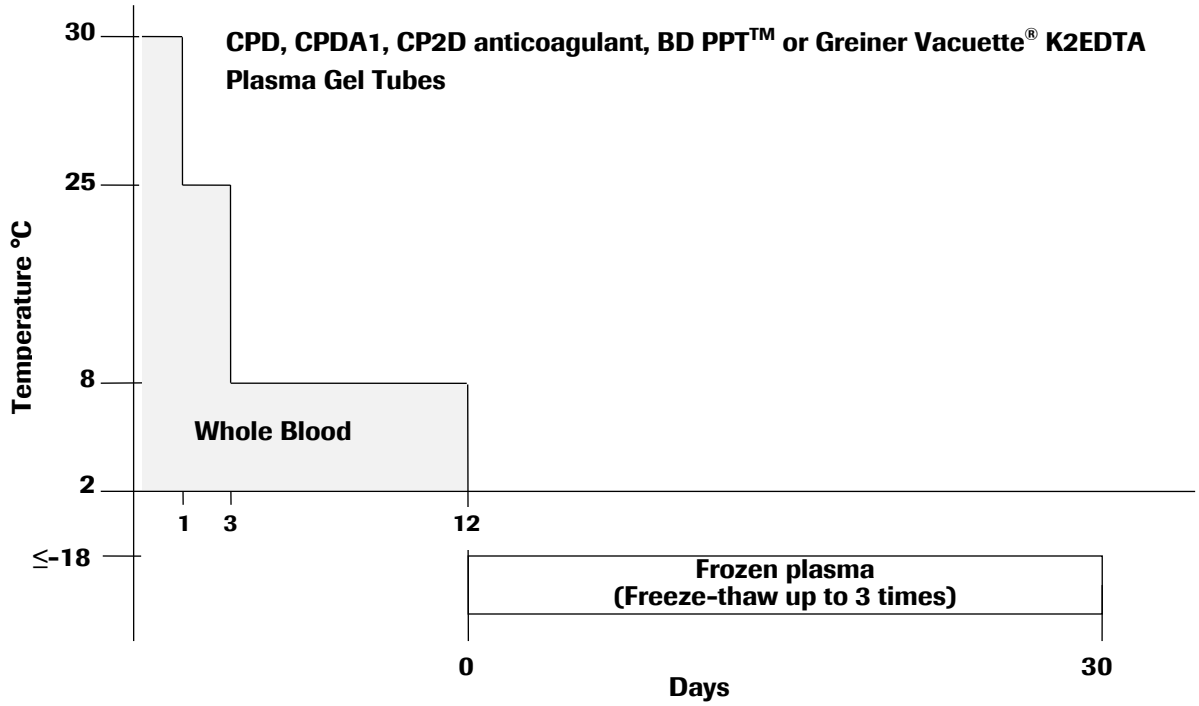
Figure 1 Sample storage conditions for samples in EDTA anticoagulant



- Blood collected in CPD, CPDA1 or CP2D anticoagulant , Becton-Dickinson EDTA Plasma Preparation Tubes (BD PPT™) or Greiner Vacuette® K2EDTA Plasma Gel Tubes 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 2.

Figure 2 Sample storage conditions for samples collected in CPD, CPDA1, CP2D, BD PPT™ and Greiner Vacuette® K2EDTA Plasma Gel tubes

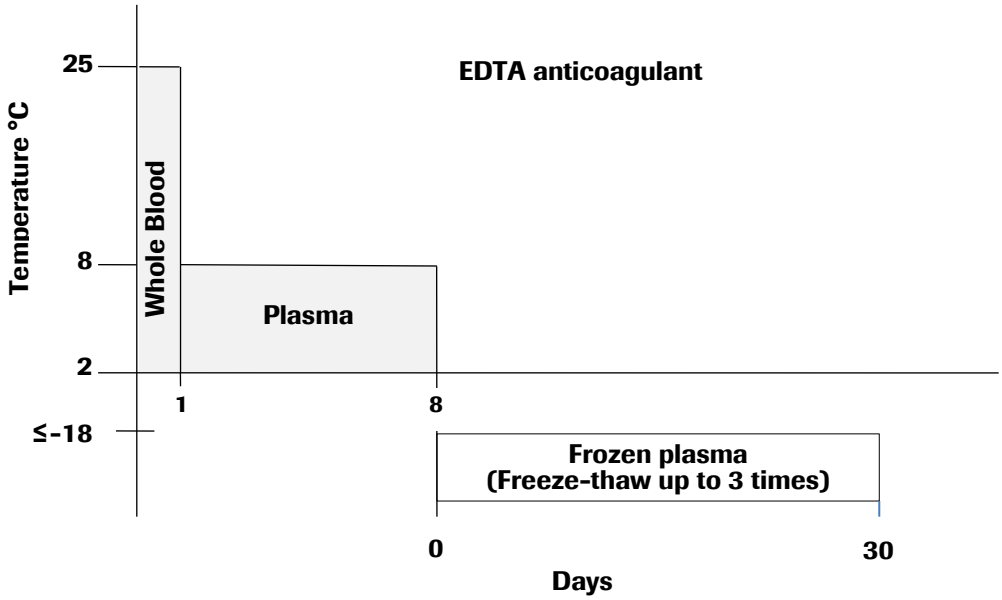


Cadaveric blood samples

- Cadaveric blood samples collected in EDTA anticoagulant tubes and/or in serum clot tubes may be used with the **cobas**® WNV test. 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 samples



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

Other than noted above, serum separated from the cells may be stored for up to 14 days at ≤-18°C with up to three freeze/thaw cycles.

- 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** software with the Hamilton MICROLAB® STAR/STARlet can be used as an optional instrument with the **cobas**® 6800/8800 systems for automated pipetting and pooling of aliquots of multiple primary samples into one pooled sample.

cobas® **Synergy** software with the Hamilton MICROLAB® STAR/STARlet IVD may be used as an accessory to the **cobas**® 5800 system for automated pipetting and pooling of aliquots of multiple primary samples into one pooled sample.

Refer to the **cobas**® p 680 instrument User Assistance or to the **cobas**® **Synergy** software User Assistance for more information.

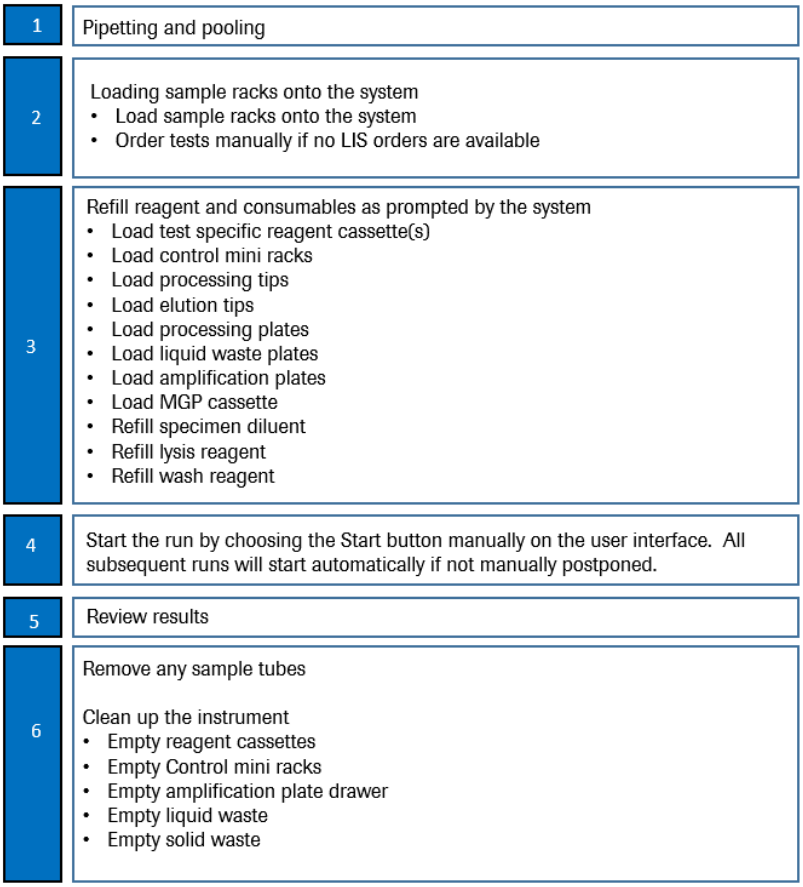
Procedural notes

- Do not use **cobas**® WNV test 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**® 5800 system User Assistance for proper maintenance of instruments.
- Refer to the **cobas**® 6800/8800 systems User Assistance for proper maintenance of instruments.
- Invalid results may be influenced by a number of contributing factors including, but not limited to, sample characteristics, interfering substances and pre-analytical workflows.

Running cobas® WNV on the cobas® 5800 system

The test procedure is described in detail in the **cobas**® 5800 system User Assistance. Figure 4 below summarizes the procedure. Refer to the **cobas**® **Synergy** software User Assistance as applicable for details on optional pooling procedures.

Figure 4 **cobas**® WNV test procedure on the **cobas**® 5800 system

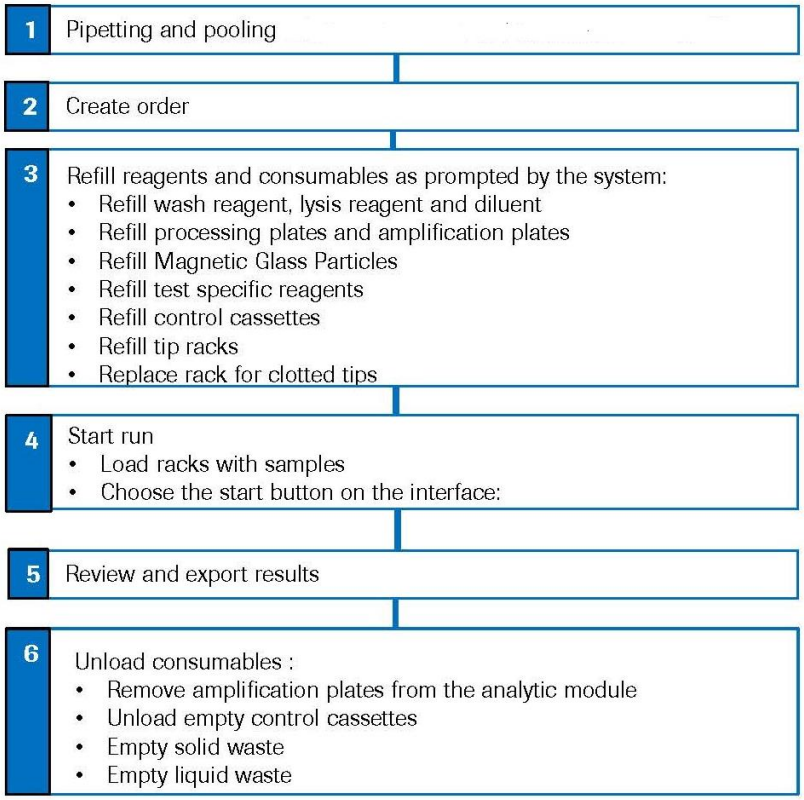


Running cobas® WNV on the cobas® 6800/8800 systems

The test procedure is described in detail in the **cobas**® 6800/8800 systems User Assistance; refer to the **cobas**® p 680 instrument User Assistance or to the **cobas**® **Synergy** software User Assistance as applicable for details on optional pooling procedures.

Figure 5 below summarizes the procedure.

Figure 5 **cobas**® WNV test procedure on the **cobas**® 6800/8800 systems



Results

The **cobas**® 5800 and **cobas**® 6800/8800 systems automatically detect WNV RNA simultaneously for the samples and controls.

Quality control and validity of results on the cobas® 5800 system

The **cobas**® 5800 system will be delivered with the default setting of controls (positive and negative) scheduled with every run, but can be configured to a less frequent control schedule, by a Roche service engineer or by contacting Roche customer technical support, based on laboratory procedures and/or local regulations.

- In the **cobas**® 5800 system and/or report, check for flags and their associated results to ensure control validity.
- The associated samples are valid if no flags appear for all controls.

Invalidation of results is performed automatically by the **cobas**® 5800 system based on negative and positive control failures.

Control results on the cobas® 5800 system

The results of the controls are shown in the **cobas**® 5800 software in the "Controls" app.

- Controls are marked with "Valid" in the column "Control result" if all Targets of the control are reported valid. Controls are marked with 'Invalid' in the column "Control result" if all or one Target of the control are reported invalid.
- Controls marked with 'Invalid' show a flag in the "Flags" column. More information on why the control is reported invalid including flag information will be shown in the detail view.
- If the positive control is invalid, repeat testing of the positive controls and all associated samples. If the negative control is invalid, repeat testing of all controls and all associated samples.

Table 11 Control flags for negative and positive controls on the **cobas**® 5800 system

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

If one of the controls is invalid, repeat testing of the respective control(s) and all associated samples.

Quality control and validity of results on the cobas® 6800/8800 systems

- 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 results on the cobas® 6800/8800 systems

Table 12 Control flags for negative and positive controls on the **cobas**® 6800/8800 systems

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**® 5800/6800/8800 systems software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid 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: one for WNV and the internal control. Final sample results for the **cobas**® WNV test are reported by the software. In addition to the overall result (**cobas**® 6800/8800 only), individual target results will be displayed in the **cobas**® 5800/6800/8800 systems software and should be interpreted as follows:

Table 13 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/or Internal Control are not meeting validity criteria

If using the **cobas**® **Synergy** software, review of the final result calculation should be performed through the **cobas**® **Synergy** software.

Additional information for interpretation of results on the cobas® 5800 system

The results of the samples are shown in the **cobas**® 5800 system. It is recommended to review results in in the **cobas**® **Synergy** software.

- Samples associated with a valid control batch (as defined by your system control configuration) are shown as 'Valid' in the "Control result" column. Samples associated with a failed control batch are shown as 'Invalid' in the "Control result" column.
- If the associated controls of a sample result are invalid, a specific flag will be added to the sample result as follows:
    - Q05D: Result validation failure because of an invalid positive control
    - Q06D: Result validation failure because of an invalid negative control
- The values in "Results" column for individual sample target result should be interpreted as shown in Table 13 above.
    - The **cobas**® 5800 system will display individual target results. The overall result will be shown only in the result view of the **cobas**® **Synergy** software.
    - For more detailed information on sample results and flags refer to the **cobas**® 5800 system User Assistance.

Interpretation of results on the cobas® 6800/8800 systems

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

- Samples are marked with "Yes" in the column 'Valid' if all requested Target Results reported valid results. Samples marked with "No" in the column 'Valid' may require additional interpretation and action.
- The values for individual sample target result should be interpreted as shown in Table 13 above.
- For more detailed information on sample results and flags refer to the **cobas**® 6800/8800 system User Assistance.

Repeat testing of individual sample(s)

Sample tubes with a final result of Invalid for the target require repeat testing.

An additional centrifugation at 600 x g for 5 minutes may help to reduce repeat invalid results for blood collected in EDTA anticoagulant, Becton-Dickinson EDTA Plasma Preparation Tubes (BD PPT™) or Greiner Vacuette® K2EDTA Plasma Gel Tubes.

Procedural limitations

- The **cobas**® WNV test 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 the **cobas**® WNV test, 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.

System equivalency/system comparison

System equivalency of the **cobas**® 5800 system with the **cobas**® 6800/8800 systems was demonstrated via equivalency studies. The results presented in these Instructions for Use are based on equivalent performance for all systems.

Non-clinical performance evaluation

Key performance characteristics

Limit of Detection (LoD)

Roche Secondary Standard/Virus Isolate

The limits of detection (LoD) of the **cobas**® WNV test 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 the **cobas**® WNV test 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 the **cobas**® WNV test kits with eight replicates per lot, for a total of 72 replicates per concentration.

For WNV lineage 1 and 2 viruses, 95% PROBIT analysis (Table 14) and 50% PROBIT analysis (Table 15) 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 intervals. The reactivity rates observed in the LoD studies for lineage 1 and 2 are summarized in Table 16 and Table 17, respectively.

Table 14 Results of 95% 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 15 Results of 50% 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	2.1	1.9	2.4
WNV Lineage 2	copies/mL	1.1	0.8	1.3

The conversion factor, traceable to the 1st WHO International Standard for West Nile Virus (WNV) RNA, NIBSC code 18/206, is 0.47 IU/copy. The conversion factor was established with the Roche Secondary Standard and can be applied to lineage 1 and lineage 2.

Table 16 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 17 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%

Reproducibility

The reproducibility of the **cobas**® WNV test on the **cobas**® 6800/8800 systems was determined using the Roche Secondary Standard for WNV Lineage 1. This study consisted of testing three panels of WNV at concentrations of approximately 18, 9 and 4.5 copies/mL. Testing was performed for the following variability components:

- day-to-day variability over three days
- lot-to-lot variability using three different reagent lots of the **cobas**® WNV test
- instrument-to-instrument variability using three different **cobas**® 8800 systems

Twenty-one replicates were tested with each of the three panels for total of 189 replicates with each reagent lot. All valid reproducibility data were evaluated by calculating the percentage of reactive test results for each concentration level across all variable components.

The limits of two-sided 95% Confidence Intervals for each Reactive Rate were calculated for each of the three levels of WNV tested across three days, three reagent lots, and three **cobas**® 8800 systems. The **cobas**® WNV test is reproducible over multiple days, reagent lots and multiple instruments. The results from reagent lot-to-lot variability are summarized in Table 18.

Table 18 cobas® WNV test reagent lot-to-lot reproducibility summary

Analyte	Concentration (Copies/mL)	Reagent lot	% Reactive (reactive/valid replicates)	Lower limit of 95% confidence interval	Upper limit of 95% confidence interval
WNV	18.0	1	98.4% (62/63)	91.5%	100.0%
		2	100% (63/63)	94.3%	100.0%
		3	100% (63/63)	94.2%	100.0%
	9.0	1	92.1% (58/63)	82.4%	97.4%
		2	98.4% (62/63)	91.5%	100.0%
		3	85.5% (53/62)	74.2%	93.1%
	4.5	1	64.5% (40/62)	51.3%	76.3%
		2	77.8% (49/63)	65.5%	87.3%
		3	79.3% (50/63)	67.3%	88.5%

Inclusivity

The performance of the **cobas**® WNV test 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 19).

For the related genus Flavivirus viruses, a total of 2 positive cultured isolates of Japanese Encephalitis Virus (JEV) were tested with 4 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 20).

Table 19 Cultured isolates of WNV Lineage 1

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

Table 20 Cultured isolates of related genus *Flavivirus* viruses

Sample Dilution	% Reactive (reactive/valid replicates tested)			
	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)

A cultured isolate of Usutu virus was also tested with 3 replicates after dilution with normal, virus-negative (WNV) human EDTA-plasma to a concentrations of 1.0E+06 copies/mL. All three replicates were detected.

Analytical specificity

The analytical specificity of the **cobas**® WNV test was evaluated for cross-reactivity with 27 microorganisms at 10^6 particles, copies, or PFU/mL, which included 19 viral isolates, six bacterial strains and one yeast isolate (Table 21). 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 the **cobas**® WNV test. The tested microorganisms do not cross-react or interfere with the **cobas**® WNV test.

Table 21 Microorganisms tested for analytical specificity

Viruses	**Flavivirus**	**Bacteria**	**Yeast**
Adenovirus 5	Dengue Virus type 1	*Escherichia coli*	*Candida albicans*
Cytomegalovirus	-	*Propionibacterium acnes*	-
Epstein-Barr Virus	-	*Staphylococcus aureus*	-
Herpes Simplex Virus type 1	-	*Staphylococcus epidermidis*	-
Herpes Simplex Virus type 2	-	*Streptococcus viridans*	-
Hepatitis A Virus Hepatitis B Virus Hepatitis C Virus	-	*Staphylococcus haemolyticus*	-
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	-	-	-
Chikungunya Virus	-	-	-
Varicella Zoster Virus	-	-	-

Plasma samples from each of the disease states (Table 22) were tested without WNV and with WNV added to a concentration of approximately 3 x LoD of the **cobas**® WNV test. These disease states do not cross-react or interfere with the **cobas**® WNV test.

Table 22 Disease states samples tested for analytical specificity

Disease state		
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 the **cobas**® WNV test. Samples containing these endogenous substances did not interfere with the sensitivity or specificity of **cobas**® WNV test.

Exogenous interference substances

Normal, virus-negative (WNV) human EDTA-plasma samples containing abnormally high concentrations of drugs (Table 23) were tested without WNV and with WNV added to a concentration of 3 x LoD of the **cobas**® WNV test. These exogenous substances did not interfere with the sensitivity or specificity of the **cobas**® WNV test.

Table 23 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

Correlation

Performance evaluation of the cobas® WNV test compared to the cobas® TaqScreen WNV Test

The performance of the **cobas**® WNV test and the **cobas**® TaqScreen WNV Test were compared using 100 individual NAT-positive EDTA-plasma samples,which were tested neat and diluted to 1:6. In addition, 100 WNVnegative EDTA-plasma samples were tested neat with both methods.

The WNV negative samples demonstrated 100% specificity by generating 100 out of 100 non-reactive results with both methods.

For positive samples, both methods were in agreement based on the McNemars's test, demonstrating that the performance of **cobas**® WNV test and **cobas**® TaqScreen WNV Test are equivalent (Table 24).

Table 24 Correlation of positive samples

Methods		WNV results	
cobas® TaqScreen WNV Test	**cobas® WNV**	**Neat**	**Diluted 1:6**
Non-reactive	Non-reactive	0	0
Reactive	Non-Reactive	0	1*
Non-reactive	Reactive	0	1*
Reactive	Reactive	100	98
Total		100	100
McNemar's Test, p-value (two-sided, α = 0.05)		1.0	1.0

* The same sample tested neat (< 100 cp/mL by National Genetic Institute using WNV RNA SuperQuant Assay) was reactive for both tests.

Whole system failure

The whole system failure rate for the **cobas**® WNV test 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 1 (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%].

Key performance characteristics – Cadaveric samples

Sensitivity

The clinical sensitivity of the **cobas**® WNV test 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 25.

Table 25 Summary of reactivity rate in cadaveric and living donor samples in EDTA plasma

Analyte	Cadaveric sample	Living donor sample
	% Reactive (Number of reactive /Number of samples tested)	% 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)	

Specificity

The specificity of the **cobas**® WNV test in cadaveric EDTA plasma and serum samples was evaluated and compared with the specificity in living donor sample by testing single replicates of 64 individual cadaveric EDTA plasma samples, of those 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), 62 individual cadaveric serum samples of those 42 individual samples were classified as moderately hemolyzed and 20 individual donor samples were classified as highly hemolyzed, 60 individual sero-negative living-donor plasma and 60 individual living-donor serum samples. The study was 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 samples from EDTA plasma and serum 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 26.

Table 26 Summary of specificity in cadaveric and living donor samples in EDTA plasma and serum

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

Reproducibility

The reproducibility of the **cobas**® WNV test 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 the **cobas**® WNV test. 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 the **cobas**® WNV test.

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 the **cobas**® WNV test

One replicate was tested with each of the 3 reagent lots over 6 days for a total of 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.

The **cobas**® WNV test is reproducible over multiple days and reagent lots for cadaveric and living donor samples. The results from reagent lot-to-lot variability are summarized in Table 27.

Table 27 cobas® WNV test 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'sExact Test ($\alpha = 0.05$)
WNV	1	Cadaveric	100.0% (120/120)	97.0%	100.0%	p-value = 1.0000
		Living donor	100.0% (120/120)	97.0%	100.0%	
	2	Cadaveric	100.0% (120/120)	97.0%	100.0%	p-value = 1.0000
		Living donor	100.0% (120/120)	97.0%	100.0%	
	3	Cadaveric	100.0% (120/120)	97.0%	100.0%	p-value = 1.0000
		Living donor	100.0% (120/120)	97.0%	100.0%	

Clinical performance evaluation

Clinical sensitivity – testing of known West Nile virus positive samples

The clinical sensitivity of the **cobas**® WNV test 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 the **cobas**® WNV test kit. Seventeen of the 530 individual clinical samples were excluded from statistical analyses, per protocol, because they did not meet the WNV viral load inclusion criteria. Thus, 513 samples were included in the analysis; however, no valid result was obtained for two of the neat samples, so only 511 neat results were included in analysis. All 513 dilute samples produced valid results.

The sensitivity of the **cobas**® WNV test with neat samples in this study was 100% (95% Confidence Interval: 99.3% to 100%) and with diluted (1:6) samples was 98.8% (95% Confidence Interval: 97.5% to 99.5%) (Table 28). The six non-reactive dilute samples were derived from neat samples with low viral titers.

Table 28 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% Confidence Interval)	
					Lower Limit	Upper Limit
Neat	511*	511	0	100	99.3	100
1:6	513	507	6	98.8%	97.5	99.5

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

Clinical specificity

The clinical specificity of the **cobas**® WNV test 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 test was calculated as the percentage (95% two-sided Confidence Interval) 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 29shows the calculation of the clinical specificity of the **cobas**® WNV test for the 63,243 evaluable donors from pooled testing. The clinical specificity of the **cobas**® WNV test from pooled testing was 100.000% (63,243/63,243; 95% Confidence Interval: 99.994% to 100.000%) in this study.

Table 29 Clinical specificity of the **cobas**® WNV test – pooled testing

cobas® **WNV Result**	**WNV Donation Status***		**Total**
	Positive	**Negative**	
WNV Reactive	0	0	0
WNV Non-Reactive	0	63,243	63,243
Total	0	63,243	63,243
Clinical Specificity (95% Confidence Interval)	**-**	**100.000% (99.994%, 100.000%)**	**-**

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

The **cobas**® WNV pool specificity for index donations was 100.000% (10,573/10,573; 95% Confidence Interval: 99.964% to 100.000%). 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 30 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 100.000% (10,823/10,823; 95% Confidence Interval: 99.965% to 100.000%) 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 30 Clinical specificity of **cobas**® WNV – individual testing

cobas® WNV Result	**WNV Donation Status***		**Total**
	Positive	**Negative**	
WNV Reactive	0	0	0
WNV Non-Reactive	0	10,823	10,823
Total	0	10,823	10,823
Clinical Specificity (95% Confidence Interval)	**-**	**100.000% (99.965%, 100.000%)**	**-**

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

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, batch = one panel + two 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 31). 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 31 Test results summarized by site, lot, day, and batch (positive panel members)

-		Site		Lot		Day		Batch	
Viral Target	**Viral Load Concen-tration**	**ID**	**% Positive Results**	**ID**	**% Positive Results**	**ID**	**% Positive Results**	**ID**	**% Positive Results**
WNV	0.5 x LoD	1	86.7% (52/60)	1	85.0% (51/60)	1	94.4% (34/36)	1	81.1% (73/90)
		2	90.0% (54/60)	2	91.7% (55/60)	2	72.2% (26/36)	2	91.1% (82/90)
		3	81.7% (49/60)	3	81.7% (49/60)	3	88.9% (32/36)	-	-
		-	-	-	-	4	86.1% (31/36)	-	-
		-	-	-	-	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)
		2	100.0% (59/59)	2	88.3% (53/60)	2	94.4% (34/36)	2	92.2% (83/90)
		3	83.3% (50/60)	3	93.2% (55/59)	3	94.3% (33/35)	-	-
		-	-	-	-	4	94.4% (34/36)	-	-
		-	-	-	-	5	94.4% (34/36)	-	-
	3.0 x LoD	1	98.3% (59/60)	1	98.3% (59/60)	1	100.0% (36/36)	1	98.9% (89/90)
		2	100.0% (60/60)	2	100.0% (60/60)	2	100.0% (36/36)	2	100.0% (90/90)
		3	100.0% (60/60)	3	100.0% (60/60)	3	97.2% (35/36)	-	-
		-	-	-	-	4	100.0% (36/36)	-	-
		-	-	-	-	5	100.0% (36/36)	-	-

Additional information

Key test features

Sample type	plasma, cadaveric plasma and cadaveric serum
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

* Tubes used for testing may have different dead volumes and require more or less minimum volume. Contact your local Roche service representative for further information.

Symbols

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

Table 32 Symbols used in labeling for Roche PCR diagnostics products

Age/DOB Age or Date of Birth	Device not for near-patient testing	QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
SW Ancillary Software	Device not for self-testing	
Assigned Range [copies/mL] Assigned Range (copies/mL)	Distributor *(Note: The applicable country/region may be designated beneath the symbol)*	SN Serial number
Assigned Range [IU/mL] Assigned Range (IU/mL)	Do not re-use	Site Site
EC REP Authorized representative in the European Community	Female	Procedure Standard Standard Procedure
BARCODE Barcode Data Sheet	For IVD performance evaluation only	STERILE EO Sterilized using ethylene oxide
LOT Batch code	GTIN Global Trade Item Number	Store in dark
Biological risks	Importer	Temperature limit
REF Catalogue number	IVD In vitro diagnostic medical device	TDF Test Definition File
CE CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device	LLR Lower Limit of Assigned Range	This way up
	Male	Procedure UltraSensitive Ultrasensitive Procedure
Collect Date Collect date	Manufacturer	UDI Unique Device Identifier
Consult instructions for use	CONTROL − Negative control	ULR Upper Limit of Assigned Range
Σ Contains sufficient for <*n*> tests	NON STERILE Non-sterile	Urine Fill Line Urine Fill Line
CONTENT Content of kit	Patient Name	Rx only For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.
CONTROL Control	Patient number	Use-by date
Date of manufacture	Peel here	
Device for near-patient testing	CONTROL + Positive control	
Device for self-testing	QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.	

Technical support

For technical support (assistance) please reach out to your local affiliate:
https://www.roche.com/about/business/roche_worldwide.htm

Manufacturer and importer

Table 33 Manufacturer and importer

Roche Molecular Systems, Inc.
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Made in USA

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68305 Mannheim, Germany

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EC REP

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Document revision

Document Revision Information	
Doc Rev. 2.0 01/2025	Addition of **cobas**® 5800 instrument throughout. Removed Rx only from front page. Updated the harmonized symbol page. Updated branding and registration information throughout. Updated **Principles of the procedure** section. Added **Reagent handling requirements for cobas® 5800 system** section. Added **Additional materials required for the cobas® 5800** section. Updated **Instrumentation and software required** section. Updated **Precautions and handling requirements** section. Updated **Instructions for use** section. Updated **Results** section and added **System equivalency/system comparison** section. Added IVD symbol. Please contact your local Roche Representative if you have any questions.

The summary of safety and performance report can be found using the following link: https://ec.europa.eu/tools/eudamed