Rx Only

cobas® Zika

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

For in vitro diagnostic use

IVD

cobas® Zika – 480	P/N: 07972466190
cobas® Zika Control Kit	P/N: 08129690190
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

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

This test is intended for use to screen donor samples for Zika virus RNA in plasma samples from individual human donors, including donors of whole blood and blood components, and 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.

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.

The test is not intended for use as an aid in diagnosis of Zika virus infection.

This test is not intended for use on samples of other body fluids.

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

Summary and explanation of the test

Background

Zika virus is an enveloped, icosahedral, single-stranded RNA arbovirus of the family Flaviviridae, genus Flavivirus. The Flaviviridae family includes West Nile virus (WNV), dengue virus, yellow fever virus, and about 70 other viruses.[1-4] Zika appears to be closely related to the Spondweni virus, which has been identified in South Africa.[1,5]

Zika virus was first isolated in 1947 from a Rhesus monkey in the Zika forest of Uganda and subsequently identified in an *Aedes africanus* mosquito captured in that forest.[1] The first three human cases were reported during a jaundice epidemic in Eastern Nigeria in 1954.[1] Zika virus has been identified in multiple countries in Africa, Asia, the Pacific Ocean, and South America.[1] A large outbreak in Brazil started in May 2015, with estimated 440,000-1,300,000 cases identified by the end of 2015.[6] By early January 2016, autochthonous Zika virus cases had been reported in more than 20 countries and territories in the Caribbean (including Puerto Rico and the U.S. Virgin Islands), Central America, and South America.[6]

Zika virus is transmitted to humans by *Aedes* mosquito species.[1] *Aedes albopictus* was described as a potential vector of Zika in 2007.[5,6,7,10,11] Zika virus may be transmitted via transfusion.[1,9,11,12-15] Zika has been detected in semen[16] and reported to have been transmitted through sexual intercourse.[16-20] Zika may also be transmitted from a pregnant woman to her fetus,[21-23] from a mother to her newborn at birth,[1,23] and through laboratory exposure.[6] Neither transmission through organ nor tissue transplantation has been documented but is theoretically possible.[6] Zika virus RNA has been detected in breast milk, but transmission through breastfeeding has not been documented.[6,23]

Limited data exist about the prevalence of Zika virus in blood donors.[1] Molecular analyses performed during the outbreak in French Polynesia in 2013 are the principal source of data.[1,11] The risk of asymptomatic persons donating blood raises concern for transfusion-related transmission and was demonstrated in the French Polynesian outbreak. There, 42 of 1,505 (2.8%) asymptomatic blood donors were positive by reverse transcription polymerase chain reaction (RT-PCR) for Zika.[1,11] Only 11 of the 42 persons infected with Zika reported a "Zika fever-like syndrome" 3 to 10 days after they donated blood.[1,18]

Zika is asymptomatic in most (estimated 80%) cases.[1,6,9,17] When symptomatic, Zika virus usually presents with non-specific, influenza-like signs and symptoms, such as mild fever, arthralgia (small joints of hands and feet), myalgia,

headache, retro-orbital pain, conjunctivitis, abdominal pain, and a maculopapular, frequently pruritic, rash; edema and lymphadenopathy may also be present.[1,5] A causal link between Zika and the occurrence of other severe neurologic symptoms, including Guillain-Barré syndrome, is still under investigation.[5,15,23] Zika infection at any time during pregnancy appears to be associated with adverse pregnancy outcomes, including congenital malformations, intrauterine growth restriction, and fetal death.[24]

The symptoms typically appear a few days after the bite of an infected mosquito and usually last 3 to 12 days.[5,14] Treatment is usually supportive, including rest, fluids, acetaminophen, and antihistamines.[5] Zika is difficult to distinguish clinically from other arboviruses, including dengue fever, chikungunya, and WNV.[1]

Serological tests (enzyme-linked immunosorbent assay [ELISA] or immunofluorescence) for Zika virus have been developed.[1] Cross-reactivity with other flaviviruses, including dengue or yellow fever, limits the utility of an IgM antibody diagnostic test.[1] In addition, antibodies may not be present in the early phase of the infection, which further reduces the suitability of a serologic test for acute infection.[5] Diagnosis of acute Zika virus infection is primarily based on detection of viral RNA from specimens via RT-PCR. The viremic period has not been established, but it is believed to be short, with direct virus detection during the first 3 to 5 days after the onset of symptoms.[1,8,25] In one study, the viral RNA concentrations were between approximately 900 and 729,000 copies/mL.[8] Viremia as high as 8.1×10^6 has been reported.[14] Most of the Zika-positive samples were from specimens collected three days or less after symptom onset, although one specimen collected on day 11 after onset had an estimated titer of 339,000 copies/mL.[8] No vaccine for Zika currently exists.[5]

Rationale for NAT testing

Zika virus can be transmitted via transfusion.[1,9,11,12-15] Most (about 80%) Zika infections are asymptomatic, so infected individuals may donate blood.[1,6,9,17] Because infected donors may not develop clinically-significant disease or remain asymptomatic, questioning blood donors about recent symptoms suggestive of Zika infection is ineffective at identifying infected donors.

Like other infectious diseases for which blood donations are screened, blood donations must be screened with a sensitive assay to detect Zika RNA so that infected units may be interdicted and discarded. The **cobas®** Zika test will offer novel capability to detect Zika RNA and thereby provide heightened protection from transfusion-transmitted Zika infection for recipients of donated blood components or products and will further improve the safety of the blood supply.

Explanation of the test

cobas® Zika is a qualitative test that is run on the **cobas®** 6800 System and **cobas®** 8800 System. **cobas®** Zika enables the simultaneous detection of Zika virus 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® Zika is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. 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.

Samples should be tested as individual samples or, optionally, can be tested in pools consisting of multiple samples. The **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 acids from the sample and added armored RNA internal control (IC) molecules (which serve as the sample preparation and amplification/detection process control) are simultaneously extracted. In addition the test utilizes two external controls: a positive and a negative control. Viral nucleic acids are released by addition of proteinase and lysis reagent to the sample. The released nucleic acids bind to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured proteins, cellular debris, and potential PCR inhibitors (such as hemoglobin) are removed with subsequent wash reagent steps and purified nucleic acids are eluted from the 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).[26-28] Any contaminating amplicons 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 amplicons are not destroyed since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

The **cobas**® Zika master mix contains detection probes which are specific for Zika virus and IC nucleic acid. The specific Zika virus and IC detection probes are each labeled with one of two unique fluorescent dyes which acts 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 detection and discrimination of the amplified Zika virus target and the IC.[29, 30] 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 Zika target and the IC are possible.

Reagents and materials

cobas® Zika reagents and controls

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

Table 1 cobas® Zika

cobas® Zika
Store at 2-8°C
480 test cassette (P/N 07972466190)

Kit components	Reagent ingredients	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.	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	38 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	38 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	14.5 mL
Zika Master Mix Reagent 2 (ZIKA MMX-R2)	Tricine buffer, potassium acetate, glycerol, 18% dimethyl sulfoxide, <0.1% Tween 20, EDTA, < 0.14% dATP, dGTP, dCTP, dUTPs, < 0.01% upstream and downstream ZIKA primers, <0.01% internal control forward and reverse primers, < 0.01% fluorescent-labeled ZIKA and internal control probes, < 0.01% oligonucleotide aptamer, < 0.01% Z05D DNA polymerase, < 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	17.5 mL

Table 2 **cobas®** Zika Control Kit

cobas® Zika Control Kit
Store at 2-8°C
(P/N 08129690190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
Zika Positive Control (Zika (+) C)	< 0.001% Synthetic (armored) Zika RNA encapsulated in MS2 bacteriophage coat protein, Normal human plasma, Zika RNA not detectable by PCR methods. 0.1% ProClin® 300 preservative	16 mL (16 x 1mL)	  Warning H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fume/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.

* Product safety labeling primarily follows EU GHS guidance

Table 3 **cobas®** NHP Negative Control Kit

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, Zika RNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative	16 mL (16 x 1mL)	  Warning H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fume/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 before reuse. P501: Dispose of contents/ container to an approved waste disposal plant.

* Product safety labeling primarily follows EU GHS guidance

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. H318: Causes serious 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 eye protection/ face protection. P304 + P340 + P312: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER or doctor/ physician if you feel unwell. 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. P501: Dispose of contents/container to an approved waste disposal plant.
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**® Zika test kit. See listing of additional materials required (Table 7).

**Product safety labeling primarily follows EU GHS guidance.

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® Zika - 480	2–8°C
cobas® Zika 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® Zika – 480	Date not passed	30 days from first usage	Max 20 runs	Max 20 hours
cobas® Zika Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 10 hours
cobas® NHP Negative Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 10 hours
cobas omni Lysis Reagent	Date not passed	30 days since loading*	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days since loading*	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days since loading*	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days since loading*	Not applicable	Not applicable

[a] Single use reagents

* 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**® Zika 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
Option for pipetting and pooling	**P/N**
cobas® **Synergy** Software Dongle	07788339001
Hamilton MICROLAB® STAR IVD	04640535001

Refer to the **cobas**® 6800/8800 Systems 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.[31,32] Only personnel proficient in handling infectious materials and the use of **cobas**® Zika and **cobas**® 6800/8800 Systems 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**® Zika 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 Zika 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**® Zika 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**® Zika 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).

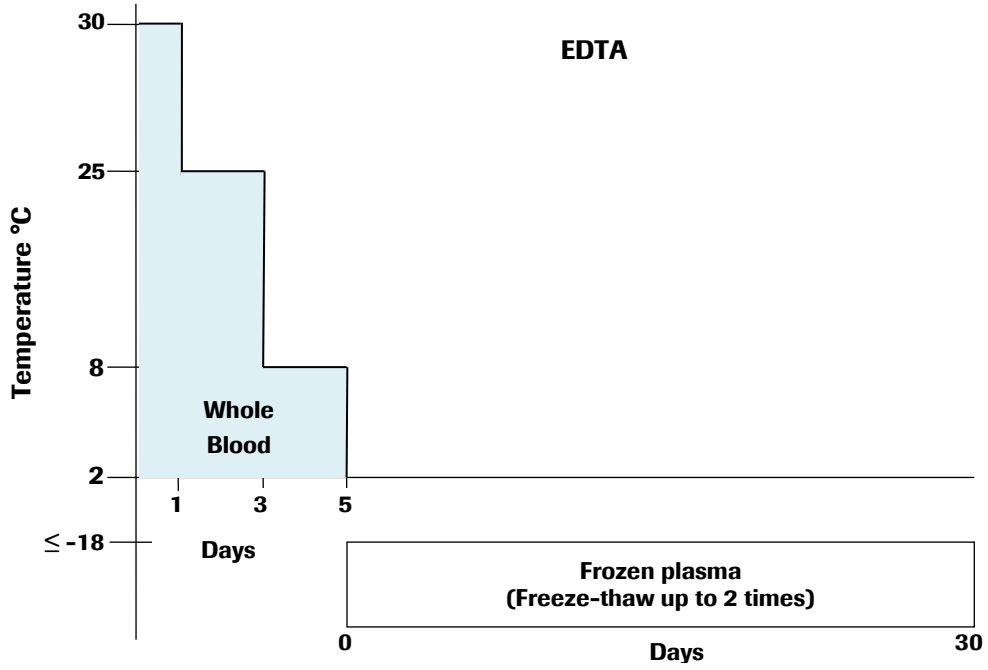
Sample collection, transport and storage

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 samples

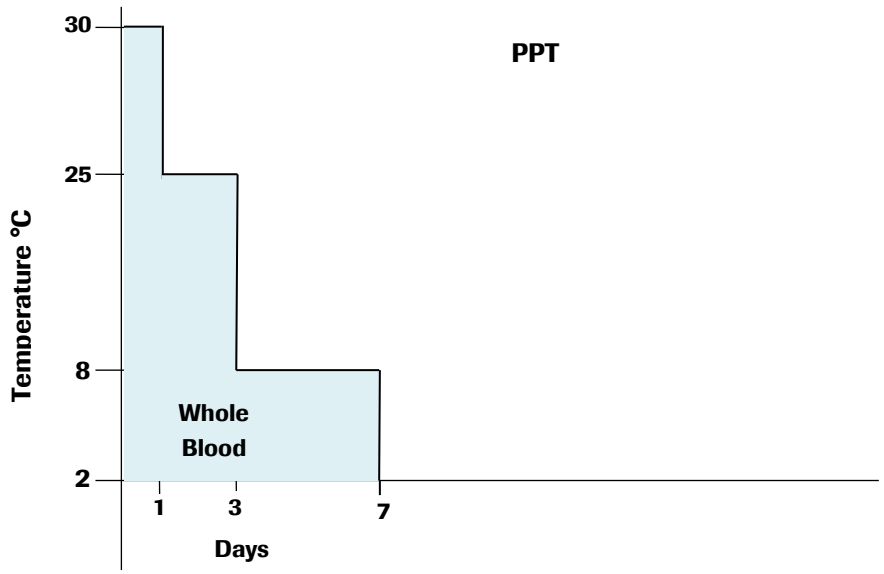
- Plasma collected in EDTA and Becton-Dickinson EDTA Plasma Preparation Tubes (BD PPT™) may be used with **cobas**® Zika. Follow the sample collection tube/bag manufacturer instructions for handling and centrifugation.
- Blood collected in EDTA may be stored for up to 5 days with the following conditions:
    - 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 two freeze/thaw cycles. Refer to Figure 1.

Figure 1 Sample storage conditions for living donor samples collected in EDTA



- Blood collected in Becton-Dickinson EDTA Plasma Preparation Tubes (BD PPT™) may be stored for up to 7 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. Refer to Figure 2.

Figure 2 Sample storage conditions for living donor samples collected in PPT



- 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.

Instructions for use

Automated sample pipetting and pooling (optional)

cobas® **Synergy** Core can be used as an optional component of the **cobas**® 6800/8800 Systems for automated pipetting and pooling of aliquots of multiple primary samples into one pooled sample. Refer to the **cobas**® **Synergy** software User Assistance for more information.

Procedural notes

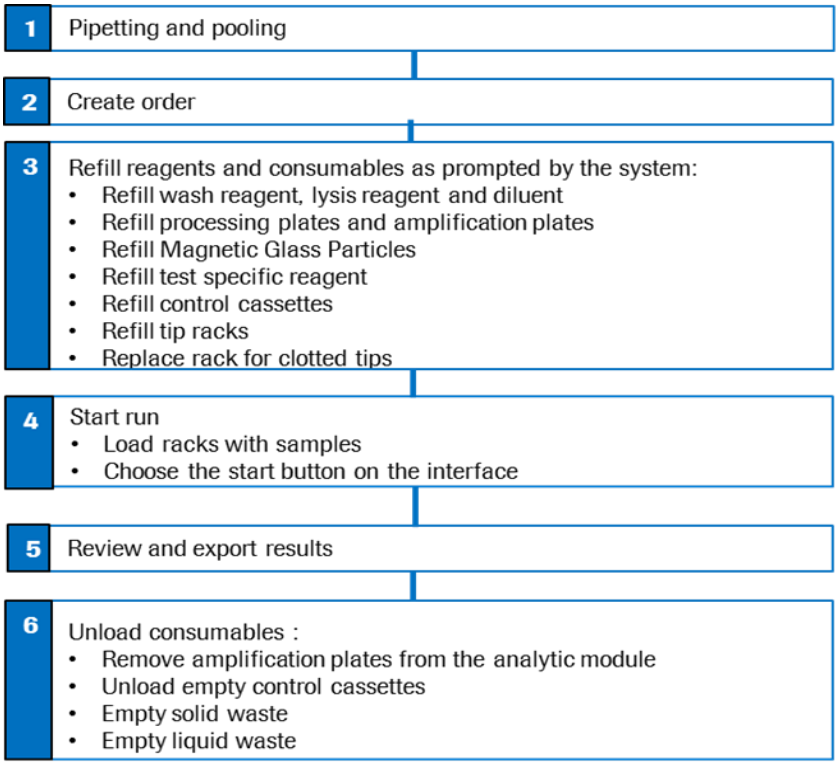
- Do not use **cobas**® Zika reagents, **cobas**® Zika Control Kit, **cobas**® NHP Negative Control Kit or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the **cobas**® 6800/8800 Systems Operator's Manual or to the **cobas**® **Synergy**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

Running cobas® Zika

The test procedure is described in detail in the **cobas**® 6800/8800 Systems Operator's Manual or refer to the **cobas**® **Synergy** software User Assistance as applicable for details on optional pooling procedures.

Figure 3 below summarizes the procedure.

Figure 3 **cobas**® Zika procedure



Results

The **cobas**® 6800/8800 Systems automatically detect Zika RNA simultaneously for the samples and controls.

Quality control and validity of results

- One negative control [(–) C] and one positive control [ZIKA (+) 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**
ZIKA (+) C	Q02	Invalid	The entire batch is assigned invalid if the result for the ZIKA (+) 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 control and the negative control of the corresponding batch are valid.

Two parameters are measured simultaneously for each sample: ZIKA and the internal control. Final sample results for the **cobas**® Zika test are reported by the software. In addition to the overall results, individual target result 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
ZIKA Non-Reactive	No target signal detected for ZIKA and IC signal detected.
ZIKA Reactive	Target signal detected for ZIKA and IC signal may be or may not be detected.
Invalid	Target and internal control signal not detected.

Procedural limitations

- **cobas**® Zika has been evaluated only for use in combination with the **cobas**® Zika 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 Zika 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.
- The **cobas**® Zika test is designed to detect Zika RNA in plasma samples, and Zika RNA may persist in certain organs and tissues, as well as other body fluids, longer than it is detectable in plasma.
- Though rare, mutations within the highly conserved regions of a viral genome covered by **cobas**® Zika, 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.

Non-clinical performance evaluation

Key performance characteristics – Living donor samples

Limit of Detection (LoD)

Roche Secondary Standard

The limit of detection (LoD) of **cobas**® Zika was determined using the Roche ZIKV Secondary Standard.

The Roche ZIKV Secondary Standard is a heat-inactivated virus culture supernatant (Strain MR766) and the titer is calculated based on the dilution factor from the stock. The stock titer was provided by the vendor (BNI, Bernhard Nocht Institute, Hamburg, Germany), and it was assigned using a serial dilution of ZIKV RNA transcript for which concentration was determined by photometric absorbance.

For the Roche ZIKV Secondary Standard, 3 independent dilution series of the viral standard were prepared with normal, virus-negative (Zika) human EDTA-plasma. Each dilution series was tested using two different lots of cobas® Zika kits with approximately 95 replicates per lot, for a total of approximately 190 replicates per concentration. For Zika virus, 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 95% confidence interval (Table 11). The reactivity rates observed in the LoD studies for Zika are summarized in Table 12.

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
Zika	cp/mL	8.1	6.1	13.6

Table 12 Reactivity rates summary for Zika in EDTA plasma

Zika RNA concentration (cp/mL)	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
16.0	190	190	100.0%	98.4%
12.0	188	190	98.9%	96.7%
8.0	180	189	95.2%	91.8%
4.0	135	189	71.4%	65.5%
2.0	94	190	49.5%	43.3%

Analytical specificity

The analytical specificity of **cobas**® Zika was evaluated for cross-reactivity with12 microorganisms at 10^5 - 10^6 particles, copies, or PFU/mL, which included 11 viral isolates and one bacterial strain (Table 13). The microorganisms were added to normal, virus-negative (Zika) human EDTA-plasma and tested with and without Zika virus added to a concentration of approximately 3 x LoD of **cobas**® Zika. The tested microorganisms do not cross-react or interfere with **cobas**® Zika.

Table 13 Microorganisms tested for analytical specificity

Viruses	Bacteria
Chikungunya Virus	*Treponema pallidum*
Dengue Virus Serotype 1	
Dengue Virus Serotype 2	
Dengue Virus Serotype 3	
Dengue Virus Serotype 4	
Japanese Encephalitis Virus	
Murray Valley Encephalitis Virus	
St. Louis Encephalitis Virus	
Usutu Virus	
West Nile Virus	
Yellow Fever Virus	

Plasma samples from each of the disease states (Table 14) were tested with and without Zika virus added to a concentration of approximately 3 x LoD of **cobas**® Zika. These disease states did not cross-react or interfere with **cobas**® Zika.

Table 14 Disease state samples tested for analytical specificity

Disease state
Human Immunodeficiency Virus
Hepatitis B Virus
Hepatitis C Virus

Analytical specificity – interfering substances

Endogenous interference substances

Plasma samples with abnormally high levels of triglycerides (up to 33.2 g/L), hemoglobin (up to 2.9 g/L), unconjugated bilirubin (up to 0.28 g/L), albumin (up to 61.4 g/L), and human DNA (up to 0.002 g/L) were tested with and without Zika virus added to a concentration of 3 x LoD of **cobas**® Zika. Samples containing these endogenous substances did not interfere with the sensitivity or specificity of **cobas**® Zika.

Exogenous interference substances

Normal, virus-negative (Zika) human EDTA-plasma samples containing abnormally high concentrations of drugs (Table 15) were tested with and without Zika virus added to a concentration of 3 x LoD of **cobas**® Zika. These exogenous substances did not interfere with the sensitivity or specificity of **cobas**® Zika.

Table 15 Concentrations of the drugs added into EDTA-plasma

Name of drug tested	Concentration
Acetaminophen	1337 µmol/L
Acetylsalicylic Acid	3657 µmol /L
Ascorbic Acid	346 µmol/L
Atorvastatin	606 µg Eq/L
Fluoxetine	11.3 µmol/L
Ibuprofen	2450 µmol/L
Loratadine	0.8 µmol/L
Nadolol	3.9 µmol/L
Naproxen	2192 µmol/L
Paroxetine	3.1 µmol/L
Phenylephrine HCL	496 µmol/L
Sertraline	2.0 µmol/L

Detection of Zika virus at LoD in clinical samples

Five Zika NAT-positive samples were singly diluted to ~13.6 copies/mL (~ 1 x LoD) into pooled negative EDTA plasma units. Twenty-one replicates were tested for each diluted sample.

All of the Zika samples diluted to near LoD were reactive for Zika and had reactive (valid) internal control (IC) results when tested using the **cobas**® Zika test (Table 16).

Table 16 Detection of Zika virus at LoD in clinical samples

Specimens	Zika RNA concentration (cp/mL)	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
5 Zika NAT-positive samples	13.6	105	105	100.0%	97.2%

Clinical performance evaluation

Reproducibility

The reproducibility of **cobas**® Zika for use on the **cobas**® 6800/8800 Systems was established by testing a twelve member panel composed of three negative plasma samples and three samples positive for Zika virus at three different concentrations (approximately 0.5 x, 1-2 x, and 3 x the LoD of **cobas**® Zika).

Operators at each of three sites performed five days of testing with each of three lots of **cobas**® Zika 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 270 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 17). This study demonstrated that **cobas**® Zika for use on the **cobas**® 6800/8800 Systems shows reproducible performance across the variables assessed (lot, site, day, batch, and within batch) for detecting Zika virus.

Table 17 Test results summarized by site, lot, day, and batch (positive panel members)

	Site		Lot		Day		Batch	
Viral Concentration	ID	% Reactive Results	ID	% Reactive Results	ID	% Reactive Results	ID	% Reactive Results
~0.5 x LoD	1	82.2% (74/90)	1	74.4% (67/90)	1	74.1% (40/54)	1	78.5% (106/135)
	2	71.9% (64/89)	2	78.7% (70/89)	2	79.6% (43/54)	2	73.7% (98/133)
	3	74.2% (66/89)	3	75.3% (67/89)	3	79.6% (43/54)	-	-
	-	-	-	-	4	73.1% (38/52)	-	-
	-	-	-	-	5	74.1% (40/54)	-	-
1-2 x LoD	1	100.0% (89/89)	1	100.0% (90/90)	1	100.0% (54/54)	1	100.0% (135/135)
	2	100.0% (90/90)	2	100.0% (89/89)	2	100.0% (53/53)	2	100.0% (134/134)
	3	100.0% (90/90)	3	100.0% (90/90)	3	100.0% (54/54)	-	-
	-	-	-	-	4	100.0% (54/54)	-	-
	-	-	-	-	5	100.0% (54/54)	-	-
~3 x LoD	1	100.0% (90/90)	1	100.0% (90/90)	1	100.0% (54/54)	1	100.0% (135/135)
	2	100.0% (90/90)	2	100.0% (90/90)	2	100.0% (54/54)	2	100.0% (135/135)
	3	100.0% (90/90)	3	100.0% (90/90)	3	100.0% (54/54)	-	-
	-	-	-	-	4	100.0% (54/54)	-	-
	-	-	-	-	5	100.0% (54/54)	-	-

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.

Clinical specificity

The clinical specificity of **cobas**® Zika was evaluated by testing individual samples from blood donations at five external laboratory sites in the Continental U.S. Four different **cobas**® Zika reagent lots were used in the study. Clinical specificity of **cobas**® Zika was calculated as the percentage (95% two-sided CI) of Zika donation status-negative donations that had **cobas**® Zika non-reactive results. There were 358,038 evaluable donations from individual testing.

Table 18 shows the calculation of clinical specificity of **cobas**® Zika for the 358,024 evaluable status-negative donations from individual testing. The clinical specificity of **cobas**® Zika from individual testing was 99.997% (358,015/358,024; 95% CI: 99.995% to 99.999%). An invalid rate of 0.09% due to internal control or other incidents was observed for individual sample results.

Table 18 Clinical specificity of **cobas**® Zika – individual testing (United States, not including the U.S. Territory of Puerto Rico)

	Zika Donation Status*		
cobas® Zika Result	**Positive**	**Negative**	**Total**
Reactive	14	9	23
Non-Reactive	0	358,015	358,015
Total	14	358,024	358,038
Clinical Specificity (95% CI)**	**-**	**99.997% (99.995%, 99.999%)**	**-**

* Donation Status was assigned based on the testing reactivity pattern observed on the index donation (initial and additional index testing) and/or based on follow-up study results.

** Clopper-Pearson Exact method

Evaluation of the sensitivity of cobas® Zika

The evaluation of the sensitivity of **cobas**® Zika was done using 25 confirmed Zika-positive clinical samples at an internal testing site. The **cobas**® Zika test detected 100% (95% CI: 86.2%-100%).

Evaluation of the yield and PPV of cobas® Zika in a Zika outbreak

Under IND, individual donation testing in the Continental U.S. began during the Zika outbreak in 2016 and identified 14 (0.004%) initially reactive and confirmed samples from 358,038 donations tested. In that same timeframe, individual testing under IND in the U.S. territory of Puerto Rico identified 275 (0.74%) initially reactive and confirmed samples from 37,041 evaluable donations tested.

The Positive Predictive Value (PPV) of **cobas**® Zika was demonstrated by confirmation of reactive samples with an alternate NAT and/or presence of anti-Zika IgM and/or **cobas**® Zika reactivity through enrollment in a follow up study. In the Continental U.S., a low prevalence area during the Zika outbreak, among 23 initially reactive blood donor samples found, 14 were confirmed to be positive, yielding a PPV of 60.9% (95% CI: 38.5%-80.3%). During the same period in the U.S. territory of Puerto Rico, an area of high prevalence, among 286 initially reactive blood donations, 275 were confirmed to be positive, yielding a PPV of 96.2% (95% CI: 93.2%-98.1%).

Evaluation of pool reactivity of cobas® Zika

Reactivity in simulated pools of 6 was calculated from the 14 and 275 confirmed positive samples in the Continental U.S. and Puerto Rico, respectively. Six out of the 14 (42.9%, 95% CI: 17.7%-71.1%) confirmed positive samples from the Continental U.S. were reactive with **cobas**® Zika in simulated pools of 6. Two hundred and fourteen out of 275 (77.8%, 95% CI: 72.4%-82.6%) confirmed positive samples from Puerto Rico were reactive with **cobas**® Zika in simulated pools of 6.

Additional information

Key test features

Sample type	Plasma
Amount of sample required	1000 µL
Amount of sample processed	850 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 19 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.		

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 20 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

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA

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Document revision

Document Revision Information	
Doc Rev. 1.0 10/2017	First Publishing.
Doc Rev. 2.0 03/2018	Addition of pooling claim. Please contact your local Roche Representative if you have any questions.
Doc Rev. 3.0 09/2018	Updated description of screening tests used for NHP screening in **Table 2**, **Table 3** and in the **Warnings and precautions** section. Updated descriptions of and added Rx Only symbol and description to the harmonized symbol page. Please contact your local Roche Representative if you have any questions.