cobas® CHIKV/DENV

For in vitro diagnostic use

cobas® CHIKV/DENV – 480	P/N: 09040650190
cobas® CHIKV/DENV Control Kit	P/N: 09040668190
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**® CHIKV/DENV for use on the **cobas**® 5800/6800/8800 systems is a qualitative in vitro test for the direct detection of chikungunya virus (CHIKV) RNA and dengue virus (DENV) serotypes 1-4 RNA in human plasma.

The test is intended for use to screen donor samples for CHIKV RNA or DENV RNA alone or to simultaneously screen for both CHIKV and DENV RNA in plasma from individual human donors, including donors of whole blood, 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 plasma may be tested in pools comprised of aliquots of individual samples.

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

This test may also be used as an aid in diagnosis for CHIKV or DENV in samples collected from individuals suspected of infection with chikungunya or dengue viruses 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

DENV is an arthropod-borne (arbovirus) RNA virus that belongs to the Flaviviridae family, which includes West Nile Virus (WNV), yellow fever virus, and about 70 other viruses.[1] Like other arboviruses, DENV is maintained in an enzootic cycle between blood-feeding mosquitoes (primarily *Aedes aegypti*) and susceptible vertebrate hosts (humans).[2,3] The World Health Organization (WHO) estimates that DENV is endemic in more than 100 countries, which includes more than 2.5 billion people at risk in the tropics and subtropical regions of the world.[2] Latin American and the Caribbean, including Puerto Rico, have experienced marked increases in DENV incidences in the past several years, raising concern for spread of *Ae. aegypti* (DENV's mosquito vector), and with it, DENV, to the United States.[4] The global burden of DENV during the 2010 pandemic is estimated at 390 million infections, which included 96 million symptomatic DENV infections and 500,000 cases of severe dengue.[5]

Most clinical DENV infections are "dengue fever," which WHO defines as fever and at least 2 other symptoms which can include chills, bone pain (often severe, from which DENV has earned the nickname "break bone fever"), myalgia, arthralgia, eye pain, rash, and easy bruising.[2] "Severe dengue" includes hemorrhagic fever and shock.[2] DENV is classified into four related, but immunologically distinct, serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. Infection with one DENV type produces lifelong immunity against that DENV serotype and short-term (≤ 2 months) cross-protection against infection with the other three DENV types.[6]

DENV is transmissible via transfusion.[2,6,7] The first documented transfusion-transmission of DENV occurred in 2002 during a local outbreak in Hong Kong; RT-PCR testing demonstrated that both donor and recipient samples were positive for DENV-1 RNA.[8,9] Clusters of transfusion-associated transmissions of DENV occurred in Singapore in 2007[10] and Puerto Rico in 2007, which included one case of transfusion-transmitted hemorrhagic fever.[1,6] During an epidemic in Brazil in 2012, 42 DENV-4 RNA-positive donations were transfused into 35 recipients and resulted in six transfusion-transmitted DENV infections.[11,12]

Most (53% to 87%) DENV infections are asymptomatic, so infected individuals may donate blood.[2] Research blood donation screening in Puerto Rico revealed a rate of 0.03% to 0.31% during recent outbreak years (2005, 2007, 2010, 2011, and 2012).[2]

A study of 39,134 blood donations collected during the 2012 Brazil DENV epidemic showed DENV-4 viremia in 0.51% of donations in Rio de Janeiro and 0.80% of donations in Recife.[11,12] Centers for Disease Control (CDC) modeling estimates a similar trend.[4] While vaccines are in development, no vaccine against DENV infection is available; treatment is supportive.[5]

CHIKV is a member of the family Togaviridae and an arthropod-borne (arbovirus) RNA virus. CHIKV is maintained in an enzootic cycle between blood-feeding mosquitoes (*Ae. aegypti* and, since at least 2005, *Ae. albopictus*) and humans.[2] CHIKV ("chikungunya" means "that which bends up" in the Makonde language of Tanzania and Mozambique) presents with similar symptoms to and during the same endemic periods as DENV, except CHIKV is characterized by severe joint pains and crippling arthritis that may cause sufferers to be unable to stand up due to intense joint pain.[2,13] Although rare, CHIKV fatalities have been reported and are typically the result of encephalitis or other encephalopathy, myocarditis, hepatitis, or multi-organ failure.[14]

CHIKV was discovered in Tanzania in 1952 and, for several decades, caused sporadic outbreaks throughout Africa and Asia.[14] Three distinct lineages of CHIKV have been identified: the West African lineage, the East Central South African (ECSA) lineage, and the Asian lineage, which is derived from the ECSA virus.[14] Since 2000, CHIKV has re-emerged to cause outbreaks of more severe forms of the disease than previously reported.[14] After an absence of 32 years in India, CHIKV produced a large outbreak, affecting 13 states, in 2006 and 2007.[14,15] A large outbreak of CHIKV in India occurred in 2006 and 2007. Several other countries in South-East Asia have experienced CHIKV outbreaks. Since 2005, India, Indonesia, Maldives, Myanmar, and Thailand have reported over 1.9 million CHIKV cases.[15] As of July 2017, Pakistan and Kenya are experiencing ongoing epidemics that began in 2016.[15]

An explosive CHIKV outbreak occurred on Reunion Island and islands of the southwest Indian Ocean, east of Africa, from late 2005 through 2007, that included 300,000 clinical cases on Reunion Island (40% of the island's population), of which 75% were symptomatic.[2,16] A mutation affecting the viral envelope protein that allowed viral replication in *Ae. albopictus* (an alternate to *Ae. aegypti*, the previously-known vector for CHIKV) was discovered in the Reunion Island outbreak; that mutation resulted in increased viral loads and virulence in the Reunion Island outbreak.[17] *Ae. albopictus* has subsequently been implicated as the mosquito vector in outbreaks in India, Northern Italy, and the Caribbean, as well. [2,18,19] While no transfusion-transmitted CHIKV infection was documented in the 2005 to 2007 Reunion Island outbreak, aggressive interventions were implemented to mitigate the risk of a transfusion-transmitted infection, which was estimated to be as high as 1,500 per 100,000 donations (1.5%).[2,16]

Sporadic cases of CHIKV have been reported in Europe. The first local outbreak (197 cases) in Europe occurred in 2007 in northeastern Italy, confirming the possibility of *Ae. albopictus*-associated outbreaks in Europe.[15] In 2014, at least 11 autochthonous cases of CHIKV were reported in Montpellier, France, caused by the invasive tiger mosquito (*Ae. albopictus*) in the vicinity of an imported case.[20]

Prior to 2013, outbreaks of CHIKV had been reported in Africa, Asia, Europe, and islands in the Indian and Pacific Oceans, but CHIKV transmission had not been documented in the Americas.[2,21] Nonetheless, the potential for CHIKV outbreaks had long been recognized because of the prevalence of the vectors and their efficiency at transmitting dengue viruses.[21] The first locally-acquired CHIKV infection in the Americas were reported from St. Martin in December 2013.[21]

CHIKV remains a concern in the Americas and the Caribbean. For 2017, through July 14, the Pan American Health Organization (PAHO) reports 58,806 suspected (28,654 confirmed) CHIKV autochthonous transmission cases in South America, the Caribbean, and North America, including 13 deaths in Brazil.[22] The majority of these cases (52,724) were reported from Brazil; the remaining cases were reported from Bolivia, Colombia, Costa Rica, El Salvador, French Guiana, Guadeloupe, Guatemala, Martinique, Nicaragua, Panama, Paraguay, Peru, Puerto Rico, St. Barthelemy, St. Martin, and Venezuela.[22]

Concern about the spread of CHIKV to the US is growing. Before 2006, CHIKV disease was rarely identified in US

travelers and no US-acquired cases had been documented.[23] From 2006 to 2013, an average of 28 people per year (range 5 to 65) in the US had positive tests for recent CHIKV infection; all were travelers visiting from or returning to the US from affected areas in Asia, Africa, or the Indian Ocean.[23] For 2014, 2,811 CHIKV disease cases were reported to ArboNET from 47 US states (excluding Alaska, Nebraska, and Wyoming), including 12 locally-transmitted cases in Florida.[23] All other cases involved travelers returning from affected areas.[23] A total of 4,710 CHIKV cases were reported in 2014 to ArboNET from US territories, including Puerto Rico, US Virgin Islands, and American Samoa.[23]

CHIKV became a nationally-reportable condition in the US in 2015. In 2015, 679 CHIKV cases were reported to ArboNET from 44 US states (all states except Delaware, Louisiana, New Mexico, South Dakota, West Virginia, and Wyoming), all in travelers returning from affected areas.[24] US territories (Puerto Rico and US Virgin Islands) reported 202 cases to ArboNET in 2015, of which all 202 were locally transmitted infections.[24] For 2016, 175 chikungunya disease cases were reported to ArboNET from 37 US states (all states except Alaska, Colorado, Idaho, Maine, Mississippi, Nevada, North Dakota, Oklahoma, Oregon, South Dakota, Vermont, West Virginia, and Wyoming).[25] All of the 175 cases occurred in travelers returning from affected areas; none were locally transmitted infections.[25]A total of 171 chikungunya disease cases were reported from US territories (all from Puerto Rico), of which 170 were locally-acquired cases and one was a travel-associated case.[25]Concern over the spread of CHIKV beyond Florida has increased with the discovery of *Ae. aegypti* mosquitoes in Los Angeles County (Commerce and Pico Rivera).[26]

Rationale for NAT testing

DENV can be transmitted via transfusion.[2,6,7] While CHIKV transfusion transmission has not been documented, the potential for transfusion-transmitted CHIKV infection is based on the transfusion transmissibility of other arboviruses, like DENV.[2] Most (53% to 87%) DENV infections, and many (approximately 25%) of CHIKV infections, are asymptomatic, so infected individuals may donate blood.[2,6,7] Because infected donors may not develop clinically-significant disease or remain asymptomatic, questioning blood donors about recent symptoms suggestive of CHIKV or DENV infection is ineffective at identifying infected donors.

Explanation of the test

cobas® CHIKV/DENV is a qualitative PCR test for the detection and discrimination of CHIKV and DENV RNA that is run on the **cobas**® 5800/6800/8800 systems. The **cobas**® CHIKV/DENV test enables simultaneous or single target screening for CHIKV and DENV RNA in a single test of an individual or pooled donation, or an individual sample for aid in diagnosis.

Principles of the procedure

The **cobas**® CHIKV/DENV test is based 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 **cobas**® 6800/8800 system software which assigns test results for all tests as non-reactive, reactive, or invalid. When using the **cobas**® 5800/6800/8800 systems**,** 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 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 if pooling is to be performed.

Nucleic acids from the sample and added armored RNA internal control (IC) molecules (which serve as a full process

control from sample preparation through amplification/detection process control) are simultaneously extracted. The IC monitors for interference that could cause false negative results. Potentially affected samples are invalidated. 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 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).[27-29] Any contaminating amplicons from previous PCR runs are eliminated 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**® CHIKV/DENV master mix contains detection probes which are specific for CHIKV, DENV and IC nucleic acid. The specific CHIKV, DENV and IC detection probes are each labeled with one of three unique fluorescent dyes which act as a reporter. Each probe also has a fourth dye which acts as a quencher. The three reporter dyes are measured at defined wavelengths, thus permitting simultaneous detection and discrimination of the amplified CHIKV and DENV targets and the IC.[30, 31] 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 three specific reporter dyes are measured at defined wavelengths, simultaneous detection and discrimination of the amplified CHIKV and DENV targets and the IC are possible.

Reagents and materials

cobas® CHIKV/DENV reagents and controls

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

Table 1 cobas® CHIKV/DENV

Store at 2-8°C
480 test cassette (P/N 09040650190)

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, glycerol EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin from Bacillus subtilis. 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
CHIKV/DENV Master Mix Reagent 2 (CHIKV/DENV 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 CHIKV and DENV primers, <0.01% internal control forward and reverse primers, < 0.01% fluorescent-labeled CHIKV, DENV 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® CHIKV/DENV Control Kit

Store at 2-8°C
(P/N 09040668190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
CHIKV/DENV Positive Control (CHIKV-DENV (+) C)	< 0.001% synthetic (armored) CHIKV and DENV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, CHIKV RNA and DENV 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, CHIKV RNA and DENV 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. H412: Harmful 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. P301 + P330 + P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
cobas® omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

* These reagents are not included in the **cobas**® CHIKV/DENV test kit. See listing of additional materials required (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 and Table 7.

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® CHIKV/DENV - 480	2–8°C
cobas® CHIKV/DENV 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® CHIKV/DENV - 480	Date not passed	90 days from first usage	Max 40 runs	Max 36 days**
cobas® CHIKV/DENV 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® CHIKV/DENV – 480	Date not passed	90 days from first usage	Max 20 runs	Max 20 hours
cobas® CHIKV/DENV 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 or Solid Waste Bag with Insert	07435967001 or 08030073001

Instrumentation and software required

The **cobas**® CHIKV/DENV 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**® CHIKV/DENV 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® p 680 instrument	06570577001
cobas® **Synergy** software electronic license (for **cobas**® 5800 system only) (Optional)	09311246001
cobas® **Synergy** Software electronic license (for **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.[32,33] Only personnel proficient in handling infectious materials and the use of **cobas**® CHIKV/DENV, 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, if applicable, 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**® CHIKV/DENV Control Kit and **cobas**® NHP Negative Control Kit contain plasma derived from human blood. Testing of normal human plasma by PCR methods also showed no detectable CHIKV RNA, and DENV 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.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- 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**® CHIKV/DENV 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**® CHIKV/DENV 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).

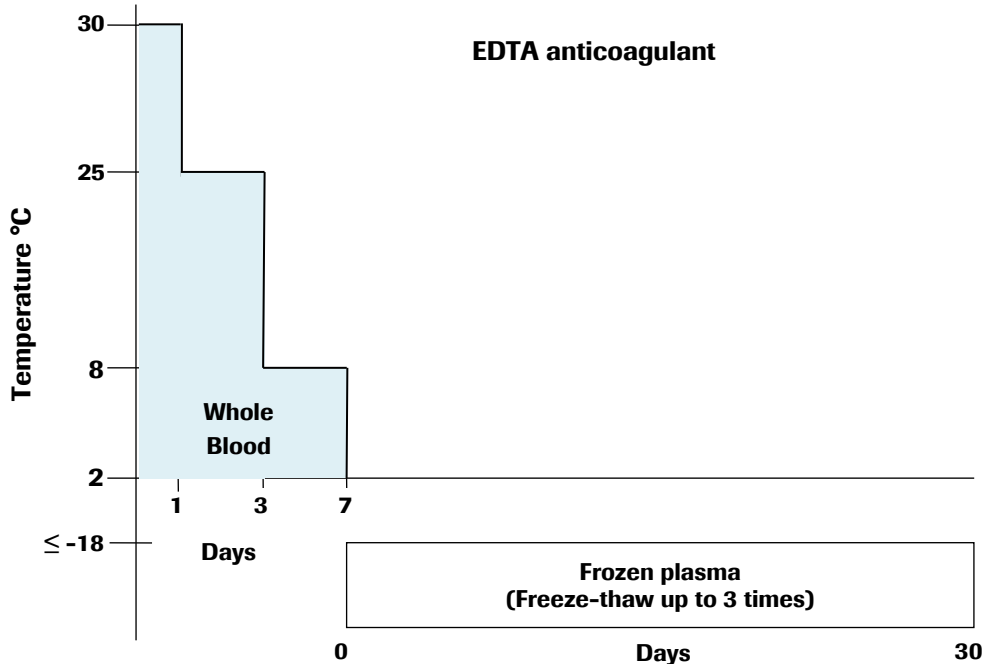
Sample collection, transport, storage, and pooling

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

Living donor and diagnostic samples

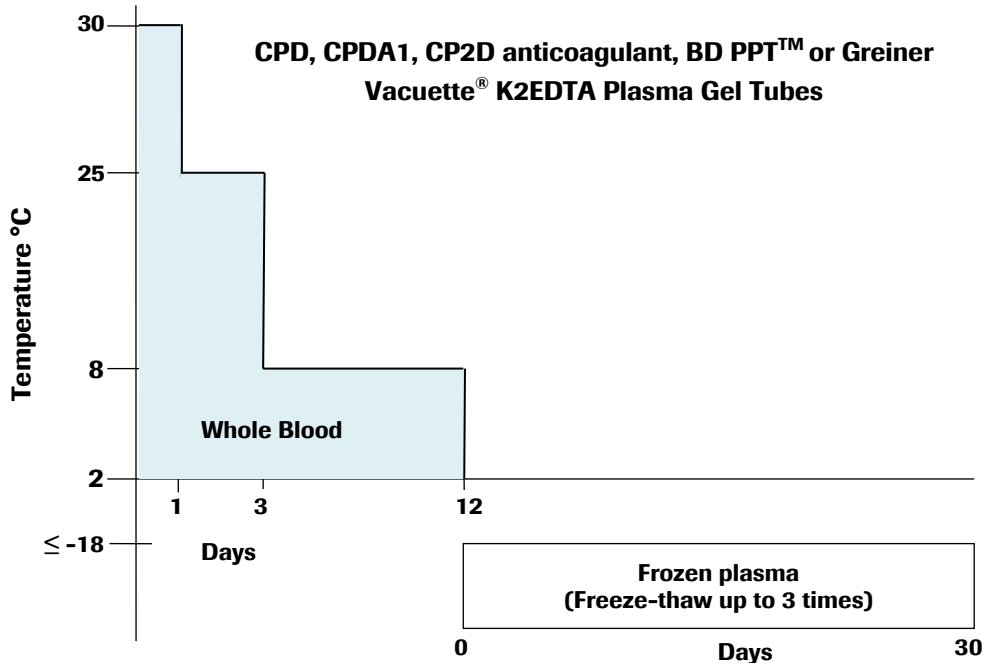
- Plasma collected in EDTA, CPD, CPDA1, CP2D may be used with **cobas**® CHIKV/DENV. Follow the sample collection tube/bag manufacturer instructions for handling and centrifugation.
- Blood collected in EDTA may be stored for up to 7 days with the following conditions:
    - Samples must be centrifuged 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. In addition, plasma separated from the cells may be stored for up to 30 days at ≤ -18°C with three freeze/thaw cycles. Refer to Figure 1.

Figure 1 Sample storage conditions for samples collected in EDTA



- Blood collected in CPD, CPDA1, CP2D, 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



- 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)

Either the **cobas**® p 680 instrument, or **cobas**® **Synergy** software with the Hamilton MICROLAB® STAR/STARlet IVD can be used as an optional component of the **cobas**® 5800/6800/8800 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.

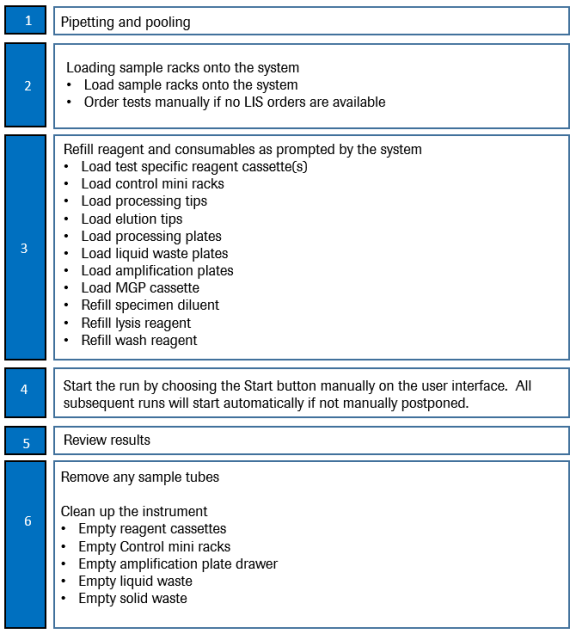
Procedural notes

- Do not use **cobas**® CHIKV/DENV test reagents, **cobas**® CHIKV/DENV 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 Operator's Manual 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® CHIKV/DENV on the cobas® 5800 system

The test procedure is described in detail in the **cobas**® 5800 system User Assistance. Figure 3 below summarizes the procedure. Refer to the **cobas**® **Synergy** software User Assistance as applicable for details on optional pooling procedures.

Figure 3 **cobas**® CHIKV/DENV test procedure on the **cobas**® **5800** system

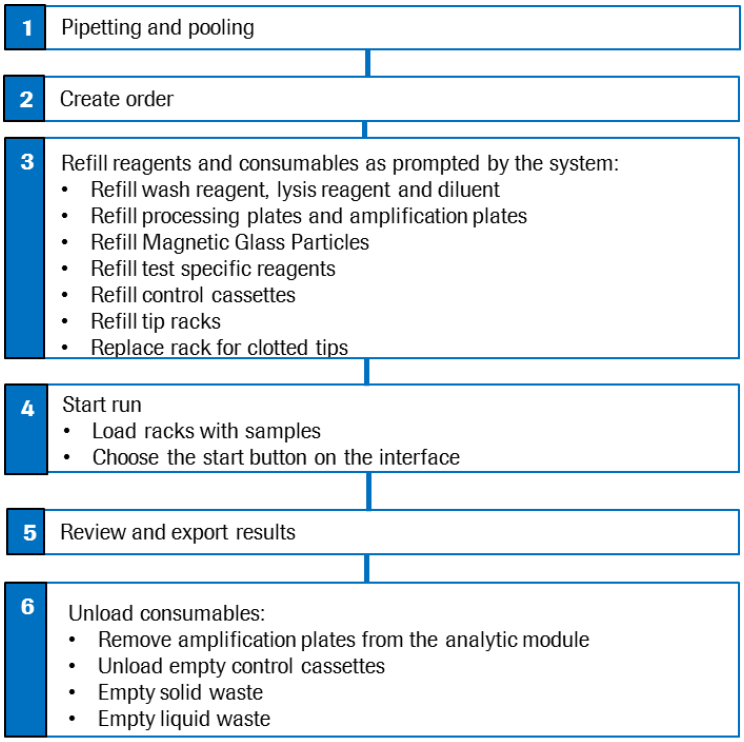


Running cobas® CHIKV/DENV on the cobas® 6800/8800 systems

The test procedure is described in detail in the **cobas**® 6800/8800 systems User Assistance or 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 4 below summarizes the procedure.

Figure 4 **cobas**® CHIKV/DENV test procedure on the **cobas**® 6800/8800 systems



Results

The **cobas**® 5800 and **cobas**® 6800/8800 systems automatically detect and discriminate CHIKV RNA and DENV RNA 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 software and/or report, check for flags and their associated results to ensure control validity.
- The associated samples are valid if no flags appear for both 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**
CHIKV-DENV (+) C	A flag is shown	Invalid	The entire batch is assigned invalid if the result for the CHIKV-DENV (+) 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, [CHIKV-DENV (+) 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

Control flags

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**
CHIKV-DENV (+) C	Q02	Invalid	The entire batch is assigned invalid if the result for the CHIKV-DENV (+) C is invalid.

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

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 control and the negative control of the corresponding batch are valid.

Three parameters are measured simultaneously for each sample: CHIKV, DENV, and the internal control. Final sample results for the **cobas**® CHIKV/DENV test are reported by the software. In addition to the overall results, individual target result (**cobas**® 6800/8800 only) 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
CHIKV Non-Reactive	No target signal detected for CHIKV and IC signal detected.
CHIKV Reactive	Target signal detected for CHIKV and IC signal may be or may not be detected.
DENV Non-Reactive	No target signal detected for DENV and IC signal detected.
DENV Reactive	Target signal detected for DENV 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 one target require repeat testing regardless of valid results for the other target.

Procedural limitations

- **cobas**® CHIKV/DENV has been evaluated only for use in combination with the **cobas**® CHIKV/DENV 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 CHIKV RNA and DENV RNA is dependent on the number of virus particles present in the sample and may be affected by sample collection, storage and handling, patient factors (i.e., age, presence of symptoms), and/or stage of infection and pool size.
- Though rare mutations within the highly conserved regions of a viral genome covered by **cobas**® CHIKV/DENV, 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

The limit of detection (LoD) of **cobas**® CHIKV/DENV was determined using the following standards:

- Roche Secondary Standard for DENV Serotype 1 (DENV-1)
- Roche Secondary Standard for DENV Serotype 2 (DENV-2)
- Roche Secondary Standard for DENV Serotype 3 (DENV-3)
- Roche Secondary Standard for DENV Serotype 4 (DENV-4)
- Roche Secondary Standard for CHIKV Genotype Asian (CHIKV-Asian)
- Roche Secondary Standard for CHIKV Genotype East Central and South African (CHIKV-ECSA)
- Armored RNA for CHIKV Genotype West African (CHIKV-WA)

The Roche DENV Secondary Standards are heat-inactivated virus culture supernatants and the titers are traceable to the 1st International Reference Panel for Dengue virus types 1 to 4 (DENV-1 BB, DENV-2 AA, DENV-3 CC and DENV-4 BB) for Nucleic Acid Amplification Techniques.

No international standards are currently available for CHIKV. The CHIKV Roche Standards(CHIKV-Asian, CHIKV-ECSA), heat-inactivated virus culture supernatants, and the armored RNA (CHIKV-WA) are traceable to CBER CHIKV RNA reference reagent (CHIKV-RR).[34]

For the Roche Secondary Standards for DENV-1 and CHIKV-Asian, 3 independent co-formulated dilution series of both viral standards were prepared with normal, virus-negative (CHIKV and DENV) human EDTA-plasma. Each dilution series was tested using three different lots of the **cobas**® CHIKV/DENV test kits with approximately 63 replicates per lot, for a total of approximately 189 replicates per concentration.

For the Roche Secondary Standards for DENV-2, DENV-3, DENV-4, CHIKV-ECSA and the armored RNA for CHIKV-WA, 3 independent series of each viral standard co-formulated for DENV-2 and CHIKV-ECSA members and individually formulated DENV-3, DENV-4 and CHIKV-WA were prepared with normal, virus-negative (CHIKV and DENV) human EDTA plasma. Each dilution series was tested using 3 different lots of the **cobas**® CHIKV/DENV test kits with approximately 42 replicates per lot, for a total of approximately 126 replicates per concentration.

For each 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 14). The reactivity rates observed in the LoD studies for each virus are summarized in Table 15 through Table 21.

Table 14 Results of PROBIT analysis on LoD data collected with viral standards in EDTA plasma

Analyte	Measuring units	LoD	Lower 95% confidence limit	Upper 95% confidence limit
DENV-1	IU/mL	0.6	0.5	0.8
DENV-2	IU/mL	1.0	0.8	1.3
DENV-3	IU/mL	1.0	0.9	1.3
DENV-4	IU/mL	0.4	0.3	0.5
CHIKV Asian	DU*/mL	6.8	5.9	8.1
CHIKV ECSA	DU*/mL	9.3	7.9	11.5
CHIKV WA	DU*/mL	7.1	6.1	8.7

* Detectable Units

Table 15 Reactivity rates summary for DENV-1 in EDTA plasma

DENV RNA concentration (IU/mL)	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
1.69	189	189	100.0%	98.4%
0.85	187	189	98.9%	96.7%
0.42	163	189	86.2%	81.4%
0.21	119	189	63.0%	56.7%
0.11	81	189	42.9%	36.7%

Table 16 Reactivity rates summary for DENV-2 in EDTA plasma

DENV RNA concentration (IU/mL)	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
3.49	126	126	100.0%	97.7%
1.75	125	126	99.2%	96.3%
0.87	116	126	92.8%	87.8%
0.44	92	126	73.0%	65.7%
0.22	60	126	47.6%	40.0%

Table 17 Reactivity rates summary for DENV-3 in EDTA plasma

DENV RNA concentration (IU/mL)	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
1.40	126	126	100.00%	97.7%
0.70	106	126	84.1%	77.8%
0.35	85	124	68.5%	61.0%
0.17	48	125	38.4%	31.1%
0.09	21	125	16.8%	11.5%

Table 18 Reactivity rates summary for DENV-4 in EDTA plasma

DENV RNA concentration (IU/mL)	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
2.40	126	126	100.0%	97.7%
1.20	126	126	100.0%	97.7%
0.60	124	126	98.4%	95.1%
0.30	116	126	92.1%	86.9%
0.15	90	126	71.4%	64.1%

Table 19 Reactivity rates summary for CHIKV-Asian in EDTA plasma

CHIKV RNA concentration (DU/mL)	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
16.0	189	189	100.0%	98.4%
8.0	188	189	99.5%	97.5%
4.0	150	189	79.4%	73.9%
2.0	94	189	49.7%	43.5%
1.0	50	189	26.5%	21.2%

Table 20 Reactivity rates summary for CHIKV-ECSA in EDTA plasma

CHIKV RNA concentration (DU/mL)	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
16.0	126	126	100.0%	97.7%
8.0	119	126	94.4%	89.8%
4.0	80	125	64.0%	56.3%
2.0	45	126	35.7%	28.6%
1.0	16	126	12.7%	8.1%

Table 21 Reactivity rates summary for CHIKV-WA in EDTA plasma

CHIKV RNA concentration (DU/mL)	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
16.0	126	126	100.0%	97.7%
8.0	122	126	96.8%	92.9%
4.0	100	126	79.4%	72.5%
2.0	54	126	42.9%	35.4%
1.0	19	126	15.1%	10.1%

Reproducibility

The reproducibility of **cobas**® CHIKV/DENV on the **cobas**® 6800/8800 systems was determined using the following standards:

- Roche Secondary Standard for DENV Serotype 1 (DENV-1)
- Roche Secondary Standard for CHIKV Genotype Asian (CHIKV-Asian)

This study consisted of testing 3 panels of co-formulated CHIKV and DENV members at concentrations of approximately 0.5 x, 1 x and 2 x the LoD of **cobas**® CHIKV/DENV for each virus. Testing was performed for the following variability components:

- day-to-day variability over 3 days
- lot-to-lot variability using 3 different reagent lots of **cobas**® CHIKV/DENV
- instrument-to-instrument variability using 3 different **cobas**® 8800 systems

Approximately 21 replicates were tested with each of the 3 panels for a total of 63 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 CHIKV and DENV tested across 3 days, 3 reagent lots, and 3 **cobas**® 8800 systems. **cobas**® CHIKV/DENV is reproducible over multiple days, reagent lots and multiple instruments. The results from reagent lot-to-lot variability are summarized in Table 22.

Table 22 cobas® CHIKV/DENV test reagent lot-to-lot reproducibility summary

Analyte	Concentration	Reagent lot	% Reactive (reactive/valid replicates)	Lower limit of 95% confidence interval	Upper limit of 95% confidence interval
DENV-1	2 x LoD	1	100% (63/63)	94.3%	100.0%
		2	100% (63/63)	94.3%	100.0%
		3	100% (63/63)	94.3%	100.0%
	1 x LoD	1	100% (63/63)	94.3%	100.0%
		2	100% (63/63)	94.3%	100.0%
		3	96.8% (61/63)	89.0%	99.6%
	0.5 x LoD	1	92.1% (58/63)	82.4%	97.4%
		2	84.1% (53/63)	72.7%	92.1%
		3	82.5% (52/63)	70.9%	90.9%
CHIKV-Asian	2 x LoD	1	100% (63/63)	94.3%	100.0%
		2	100% (63/63)	94.3%	100.0%
		3	100% (63/63)	94.3%	100.0%
	1 x LoD	1	100% (63/63)	94.3%	100.0%
		2	100% (63/63)	94.3%	100.0%
		3	98.4% (62/63)	91.5%	100.0%
	0.5 x LoD	1	77.8% (49/63)	65.5%	87.3%
		2	87.3% (55/63)	76.5%	94.4%
		3	73.0% (46/63)	60.3%	83.4%

Genotype verification

The performance of **cobas**® CHIKV/DENV to detect all 4 serotypes of DENV and all 3 genotypes of CHIKV was determined by testing a total of 43 unique clinical samples, 2 cultured isolates and 1 armored RNA (aRNA) with known serotypes/genotypes. All 43 clinical samples were tested neat and after dilution with normal, virus-negative (CHIKV and DENV) human EDTA-plasma to 4 x LoD of **cobas**® CHIKV/DENV.

All clinical samples and cultured isolates were detected at neat and at 4 x LoD (Table 23).

Table 23 CHIKV/DENV clinical samples, cultured isolates and armored RNA

Target	Genotype/ Serotype	Samples	% Reactive (reactive/samples tested) neat	% Reactive (reactive/samples tested) diluted to 4x LoD
CHIKV	Asian	10 clinical samples	100% (10/10)	100% (10/10)
	ECSA	1 cultured isolate	100% (1/1)	100% (1/1)
	West African	1 aRNA	100% (1/1)	100% (1/1)
DENV	1	10 clinical samples	100% (10/10)	100% (10/10)
	2	10 clinical samples	100% (10/10)	100% (10/10)
	3	3 clinical samples, 1 cultured isolate	100% (4/4)	100% (4/4)
	4	10 clinical samples	100% (10/10)	100% (10/10)

Analytical specificity

The analytical specificity of **cobas**® CHIKV/DENV was evaluated for cross-reactivity with 31 microorganisms at 10^5 - 10^6 copies, genome equivalents, IU or CFU/mL, which included 24 viral isolates, six bacterial strains and one yeast isolate (Table 24). The microorganisms were added to normal, virus-negative (CHIKV and DENV) human EDTA-plasma and tested with and without CHIKV and DENV (co-formulated) added to a concentration of approximately 3 x LoD of **cobas**® CHIKV/DENV. The tested microorganisms do not cross-react or interfere with **cobas**® CHIKV/DENV.

Table 24 Microorganisms tested for analytical specificity

Viruses	Flaviviruses	Bacteria	Yeast
Adenovirus type 5	Japanese Encephalitis Virus	*Escherichia coli*	*Candida albicans*
Cytomegalovirus	Murray Valley Encephalitis Virus	*Propionibacterium acnes*	
Epstein-Barr Virus	St. Louis Encephalitis Virus	*Staphylococcus aureus*	
Hepatitis A Virus	Usutu Virus	*Staphylococcus epidermis*	
Hepatitis B Virus	West Nile Virus	*Streptococcus viridans*	
Hepatitis C Virus	Yellow Fever Virus	*Staphylococcus haemolyticus*	
Hepatitis E Virus	Zika Virus		
Hepatitis G Virus			
Herpes Simplex Virus type 1			
Herpes Simplex Virus type 2			
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 6A			
Influenza Virus A			
Varicella Zoster Virus			

Plasma samples from each of the disease states (Table 25) were tested with and without CHIKV and DENV (co-formulated) added to a concentration of approximately 3 x LoD of **cobas**® CHIKV/DENV for each virus. These disease states do not cross-react or interfere with **cobas**® CHIKV/DENV.

Table 25 Disease state samples tested for analytical specificity

Disease state		
Adenovirus type 5	Hepatitis C Virus	Human T-cell lymphotropic Virus type I
Cytomegalovirus	Hepatitis E Virus	Human T-cell lymphotropic Virus type II
Epstein-Barr Virus	Herpes Simplex Virus type 1	Parvovirus B19
Hepatitis A Virus	Herpes Simplex Virus type 2	West Nile Virus
Hepatitis B Virus	Human Immunodeficiency Virus (HIV-1) Group M	

Analytical specificity – interfering substances

Endogenous interference substances

Plasma samples with abnormally high levels of triglycerides (up to 33.0 g/L), hemoglobin (up to 2.0 g/L), unconjugated bilirubin (up to 0.20 g/L), albumin (up to 60.0 g/L), and human DNA (up to 0.002 g/L) were tested with and without CHIKV and DENV (co-formulated) added to a concentration of 3 x LoD of **cobas**® CHIKV/DENV. Samples containing these endogenous substances did not interfere with the sensitivity or specificity of **cobas**® CHIKV/DENV.

Exogenous interference substances

Normal, virus-negative (CHIKV and DENV) human EDTA-plasma samples containing abnormally high concentrations of drugs (Table 26) were tested with and without CHIKV and DENV (co-formulated) added to a concentration of 3 x LoD of **cobas**® CHIKV/DENV for each virus. These exogenous substances did not interfere with the sensitivity or specificity of **cobas**® CHIKV/DENV.

Table 26 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

Correlation

Performance evaluation of cobas® CHIKV/DENV compared to the RealStar® Chikungunya RT-PCR Kit 2.0 test and RealStar® Dengue RT-PCR Kit 2.0 test

The performance of **cobas**® CHIKV/DENV was compared to the RealStar® Chikungunya RT-PCR Kit 2.0 test and the RealStar® Dengue RT-PCR Kit 2.0 test (Altona Diagnostics) using 100 individual CHIKV NAT-positive samples, 100 individual DENV NAT-positive samples and 100 CHIKV and DENV negative plasma samples.

The negative samples were tested neat with **cobas**® CHIKV/DENV, RealStar® Chikungunya RT-PCR Kit 2.0 test and the RealStar® Dengue RT-PCR Kit 2.0 test, the positive samples were tested neat with **cobas**® CHIKV/DENV and the corresponding RealStar® test.

The seronegative samples demonstrated 100% specificity by generating 100 out of 100 non-reactive results with all three methods.

For positive samples, **cobas**® CHIKV/DENV is more sensitive for CHIKV and DENV than RealStar® Chikungunya RT-PCR Kit 2.0 test and the RealStar® Chikungunya RT-PCR Kit 2.0 test. The methods were not in agreement based on the McNemars's test (Table 27).

Table 27 Correlation of positive samples (neat)

Methods		Individual viral target results	
RealStar® CHIKV RT-PCR Kit 2.0 test RealStar® DENV RT-PCR Kit 2.0 test	**cobas® CHIKV/DENV**	**CHIKV**	**DENV**
Non-reactive	Non-reactive	1	0
Reactive	Non-Reactive	0	0
Non-reactive	Reactive	14	19
Reactive	Reactive	85	81
Total		100	100
McNemar's Test, p-value (two-sided, α=0.05)		0.0001	0.0000

Whole system failure

The whole system failure rate for **cobas**® CHIKV/DENV was determined by testing 100 replicates of EDTA plasma spiked with CHIKV and DENV (co-formulated). 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**® 6800 system with **cobas**® p 680 instrument (pipetting and pooling).

The results of this study determined that all replicates were reactive for each target, 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%].

Clinical performance evaluation

Clinical (diagnostic) sensitivity

The evaluation of the diagnostic sensitivity of **cobas**® CHIKV/DENV was done using 111 confirmed Chikungunya-positive clinical samples and 134 confirmed Dengue-positive clinical samples tested neat at an internal testing site. The **cobas**® CHIKV/DENV test detected 100% (111/111; 95% two-sided Confidence Interval (CI): 96.7%-100%) for Chikungunya virus and 100% (134/134; 95% CI: 97.3%-100%) for Dengue virus (Table 28).

Table 28 Diagnostic sensitivity of CHIKV and DENV known positive samples (neat)

Target	Number of Samples Tested	Number of Samples Reactive	Number of Samples Non-Reactive	Diagnostic Sensitivity	95% Exact CI
CHIKV	111	111	0	100%	(96.7%, 100%)
DENV	134	134	0	100%	(97.3%, 100%)

Clinical (diagnostic) specificity

The clinical (diagnostic) specificity of **cobas**® CHIKV/DENV was evaluated by testing individual samples from blood donations from both mainland US and Puerto Rico, CHIKV/DENV non endemic and endemic regions respectively, at one external laboratory site in the US. One **cobas**® CHIKV/DENV reagent lot was used for both studies. Clinical specificity of **cobas**® CHIKV/DENV was calculated as the percentage (95% two-sided CI) of CHIKV/DENV donation status-negative donations that had **cobas**® CHIKV/DENV non-reactive results. There were 10,528 evaluable donations from the non endemic region and 1,056 evaluable donations from the endemic region.

The specificity of the **cobas**® CHIKV/DENV test was 100% for both the CHIKV and DENV targets in blood donations overall and separately from non endemic and endemic regions (Table 29).

Table 29 Clinical (diagnostic) specificity of **cobas**® CHIKV/DENV – Individual donation testing

Endemicity	Target	Total Number of Status-Negative Donations	cobas® CHIKV/DENV Reactive	cobas® CHIKV/DENV Non-Reactive	Diagnostic Specificity Estimate in Percent (95% Exact CI)
Non Endemic	**CHIKV**	10,528	0	10,528	100.000 (99.965, 100.000)
Non Endemic	**DENV**	10,528	0	10,528	100.000 (99.965, 100.000)
Endemic	**CHIKV**	1,056	0	1,056	100.000 (99.651, 100.000)
Endemic	**DENV**	1,056	0	1,056	100.000 (99.651, 100.000)
Overall	**CHIKV**	11,584	0	11,584	100.000 (99.968, 100.000)
Overall	**DENV**	11,584	0	11,584	100.000 (99.968, 100.000)

Additional information

Key test features

Sample type	plasma
Minimum amount of sample required	1000 µL*
Amount of sample processed	850 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 30 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 31 Manufacturer and importer

Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876, USA
www.roche.com

Made in USA

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Trademarks and patents

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EC REP Roche Diagnostics GmbH
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IVD

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Document revision

Document Revision Information	
Doc Rev. 1.0 10/2024	First Publishing
Doc Rev 2.0 03/2025	Addition of **cobas**® 5800 instrument throughout. Updated branding and registration information throughout. Updated the CHIKV/DENV 480T kit and omni reagent hazard information to match BSI approved version. 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. Please contact your local Roche Representative if you have any questions.
Doc Rev 3.0 07/2025	Updated Table 7 for the CHIKV/DENV 480 in the Open-kit stability from 60 days to 90 days for **cobas**® 6800/8800 systems. 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