cobas® HEV

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

For in vitro diagnostic use

cobas® HEV – 96	P/N: 07001045190
cobas® HEV Control Kit	P/N: 07001100190
cobas® NHP Negative Control Kit	P/N: 07002220190

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Intended use

The **cobas**® HEV test is a qualitative *in vitro* nucleic acid amplification test for the direct detection of hepatitis E virus (HEV) RNA (genotypes 1-4) in human plasma.

This test is intended for use to screen donor samples for HEV RNA in plasma samples from individual human donors, including donors of whole blood, blood components (red cells, platelets, and plasma), and other living donors. 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 on samples of cord blood.

This test is not intended for use as an aid in diagnosis for HEV.

Summary and explanation of the test

Background: Screening of blood for transfusion-transmitted viral infections

Hepatitis E virus (HEV), a small, non-enveloped, RNA virus belonging to the Hepevirus genus (family Hepeviridae), is a human pathogen with a worldwide distribution.[1] The virus consists of an icosahedral particle that encloses a positive-sense, single-stranded RNA genome of 7.2kb.[2] Four major HEV genotypes, representing a single serotype, have been identified in humans and animals, including domestic pigs, wild boar, deer, and rodents.[1,3,4]

Molecular characterization of various HEV strains circulating among humans and animals has led to the recognition of four major genotypes.[1] Genotype 1, which occurs mainly in Asia, and genotype 2, which occurs in Africa and Mexico, are restricted to humans and transmitted via contaminated water in developing countries.[1,5] Genotypes 3 and 4 infect humans, pigs, and other mammalian species and cause sporadic cases of autochthonous HEV in both developing and developed countries.[6] Genotype 3 is the only genotype currently identified as the cause of autochthonous infection in the U.S.[7] and is the cause of the vast majority of infections in Europe, New Zealand, and North America.[1,8-12] Genotypes 3 and 4 are both present in Japan.[1] HEV genotypes 1, 3, and 4 are endemic to China.[1] Acute Hepatitis E is more common than Hepatitis A in China, France, the U.K., and Japan.[1]

The main mode of HEV transmission is the fecal-oral route through contaminated drinking water,[1] although food-borne transmission from consumption of undercooked or raw pork, organ meat, or shellfish, as well as zoonotic transmission as a result of contact with infected swine, domestic, or wild animals have been reported.[7,13] The full range of reservoirs for HEV is unknown.[1]

HEV infection usually causes a mild or subclinical infection with a self-limiting illness that lasts 4 to 6 weeks.[1,8-11,17,18] The symptoms are very similar to those of other forms of viral hepatitis infection, particularly Hepatitis A, with fatigue, jaundice, fever, malaise, nausea, vomiting, anorexia, and abdominal pain.[1] Patients often present with elevated alanine transaminase levels (~1,500 IU/L) and many present with jaundice.[1] HEV infections may occasionally be more severe and result in fulminant hepatic failure, particularly in pregnant woman, where the mortality rate can reach 10% to 25%; infants and children under 2 years of age, individuals with underlying liver disease (e.g., cirrhosis), and immunocompromised persons.[1,19-23] HEV infection causes more than 3 million symptomatic cases of acute Hepatitis E worldwide each year, which results in approximately 70,000 deaths annually.[24] The overall mortality rate ranges from 0.2% to 4.0%.[1,25] Most deaths from genotype 3 (HEV3) infection result from acute or subacute liver failure in patients with pre-existing liver disease, such as alcohol-related liver disease.[1,22,26,27]

HEV3 causes chronic infection, including up to 60% of infected immunosuppressed individuals, about 10% of whom develop cirrhosis.[1] Chronic infection is defined as persistent HEV RNA in serum or stool for 6 months or more.[1] Most cases occur in solid-organ transplant recipients, although infection in individuals with hematologic disorders receiving transfusions and chemotherapy and a few individuals with HIV have also been reported.[28-34] Chronic infection has not been reported with HEV1 or HEV2.[1]

HEV infection has also been associated with neurologic syndromes, including Guillain-Barré syndrome, Bell's palsy, acute transverse myelitis, acute meningoencephalitis, ataxia, and encephalitis; the neurologic symptoms typically resolve in patients who clear the virus.[1] Membranoproliferative and membranous glomerulonephritis, acute pancreatitis, and severe thrombocytopenia have been reported during acute HEV infection, although the pathophysiologic mechanisms and causal

relation, if any, have not been established.[1] Ribavirin therapy has been shown to be an effective treatment for acute severe HEV3 infection, and transplant recipients with chronic HEV infection are typically treated with reduction of immunosuppression (especially drugs that target T cells), interferon-α, and ribavirin.[1]

Rationale for NAT testing

Like other hepatitides, HEV can be transmitted via transfusion of blood or blood products. Post-transfusion Hepatitis E has been reported in many countries.[1,34-39] The seropositive rate for HEV among the world's blood donors has been reported to vary from 0.4%–20.6%.[40-48] Asymptomatic HEV infections occur at a high rate around the world and, due to the prevalence of the virus, many blood donors may be infected and transmit the virus to recipients of their blood products. For example, a recent study of British blood donors showed 11% of donor sera was HEV IgG reactive, indicating past infection, and 0.7% of donor sera was IgM reactive, indicating acute infection.[46] In addition, 0.7% of plasma minipools from English donors contained HEV RNA.[47] A study of Chinese blood donors revealed similar results: 32.6% of donor sera was IgG reactive; 0.94% of donor sera was IgM reactive; and 0.07% of donations demonstrated HEV viremia.[48] A global investigation of plasma fractionation pools reported 10% of pools tested were HEV-RNA positive.[48,49]

Explanation of the test

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

Principles of the procedure

The **cobas**® HEV test 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 can either be tested individually or tested in pools consisting of multiple samples. The **cobas p** 680 instrument 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).[50-52] 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**® HEV master mix contains detection probes which are specific for HEV and IC nucleic acid. The specific HEV and IC detection probes are each labeled with one of two unique fluorescent dyes which act as a reporter. Each probe also has a second dye which acts as a quencher. The two reporter dyes are measured at defined wavelengths, thus permitting simultaneous detection and discrimination of the amplified HEV target and the IC.[53,54] The fluorescent signals of the intact probes are 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 HEV target and the IC are possible.

Reagents and materials

cobas® HEV reagents and controls

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

Table 1 cobas® HEV test

cobas® HEV test
Store at 2-8°C
96 test cassette (P/N 07001045190)

Kit components	Reagent ingredients	Quantity per kit 96 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase EUH210: Safety data sheets available on request. EUH208: May produce an allergic reaction. Contains: Subtilisin, 9014-01-1	13 mL
Internal Control (IC)	Tris buffer, < 0.05% EDTA, < 0.001% internal control armored RNA construct (non-infectious RNA encapsulated in MS2 bacteriophage), < 0.002% Poly rA RNA (synthetic), < 0.1% sodium azide	13 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	13 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	5.5 mL
HEV Master Mix Reagent 2 (HEV MMX-R2)	Tricine buffer, potassium acetate, glycerol, 18% dimethyl sulfoxide, Tween 20, EDTA, < 0.06% dATP, dGTP, dCTP, < 0.14% dUTP, < 0.01% upstream and downstream HEV and internal control primers, < 0.01% fluorescent-labeled HEV probes, < 0.01% fluorescent-labeled internal control probe, < 0.01% oligonucleotide aptamer, < 0.01% ZO5D DNA polymerase, < 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	6 mL

Table 2 **cobas**® HEV Control Kit

cobas® HEV Control Kit
Store at 2-8°C
(P/N 07001100190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning
HEV Positive Control (HEV (+) C)	< 0.001% synthetic (armored) HEV RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative	16 mL (16 x 1 mL)	  Warning H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fumes/gas/mist/ vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P302+ P352: IF ON SKIN wash with plenty of soap and water. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P363: Wash contaminated clothing before reuse.

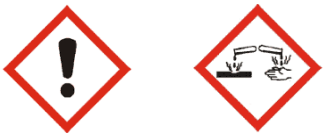
Table 3 **cobas**® NHP Negative Control Kit

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, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative	16 mL (16 x 1 mL)	  Warning H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fumes/gas/mist/vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P302+ P352: IF ON SKIN wash with plenty of soap and water. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P363: Wash contaminated clothing before reuse.

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 875mL	 Danger H302: Harmful if swallowed. H318: Causes serious eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P301 + P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P264: Wash skin thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P273: Avoid release to the environment. P280: Wear protective gloves/eye protection/face protection. P305+P351+P338: IF IN EYES Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310: Immediately call a POISON CENTER or doctor/Physician if you feel unwell. P330: Rinse mouth
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2L	Not applicable

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

Reagent storage and handling requirements

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® HEV – Test 96	2–8°C
cobas® HEV 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® HEV – Test 96	Date not passed	30 days since first usage	Max 10 runs	Max 8 hours
cobas® HEV Control Kit	Date not passed	Not applicable	Not applicable	Max 8 hours
cobas® NHP Negative Control Kit	Date not passed	Not applicable	Not applicable	Max 8 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

* 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**® HEV analysis package shall be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

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
cobas p 680 Instrument (optional for pipetting and pooling)	06578624001
Sample Supply Module	06301037001

Refer to the **cobas**® 6800/8800 Systems Operator's Manual and **cobas p** 680 instrument Operator's Manual for additional information for 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-A3.[38,39] Only personnel proficient in handling infectious materials and the use of the **cobas**® HEV test, **cobas**® 6800/8800 Systems and **cobas p** 680 instrument (optional) 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**® HEV Control Kit and **cobas**® NHP Negative Control Kit contain plasma derived from human blood. The source material has been tested by licensed antibody tests and found non-reactive for the presence of antibody to HCV, antibody to HIV-1/2, HBsAg, and antibody to HBc. Testing of normal human plasma by PCR methods also showed no detectable HEV RNA, HIV-1 (Groups M and O) RNA, HIV-2 RNA, HCV RNA, HBV DNA, WNV RNA, or CMV DNA. 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.
- 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**® HEV test kits, **cobas omni** MGP Reagent, and **cobas omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- 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**® HEV 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 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 6800/8800 instruments, follow the instructions in the **cobas**® 6800/8800 Systems Operator's Manual to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport, storage, and pooling

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

Store all donor samples at specified temperatures.

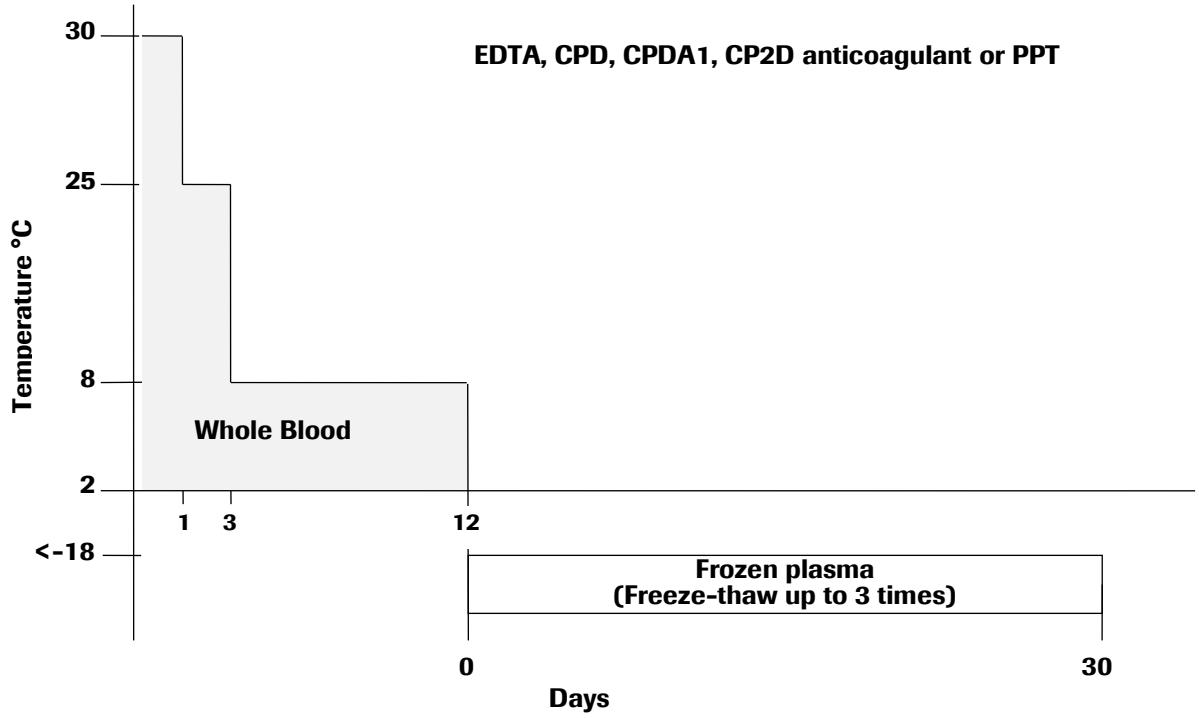
Sample stability is affected by elevated temperatures.

Living donor samples

- Plasma collected in EDTA, CPD, CPDA1, CP2D and 4% Sodium Citrate (Apheresis plasma) anticoagulant may be used with the **cobas**® HEV test. Follow the sample collection tube/bag manufacturer instructions for handling and centrifugation.
- Blood collected in EDTA, CPD, CPDA1, CP2D anticoagulant or Becton-Dickinson EDTA Plasma Preparation Tubes (BD PPT™) may be stored for up to 12 days with the following conditions:
    - Samples must be centrifuged within 72 hours of draw.
    - For storage above 8°C, samples may be stored for 72 hours at up to 25°C, and up to 30°C for 24 hours during the 72 hours.

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

Figure 1 Sample storage conditions



- Apheresis plasma collected in 4% Sodium Citrate anticoagulant may be stored for up to 30 days at 2-8°C.
  - 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.
- 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)

The **cobas p** 680 instrument is an optional component of the **cobas**® 6800/8800 Systems used for automated pipetting and pooling of aliquots of multiple primary samples into one pooled sample. Refer to the **cobas p** 680 instrument Operator's Manual for more information.

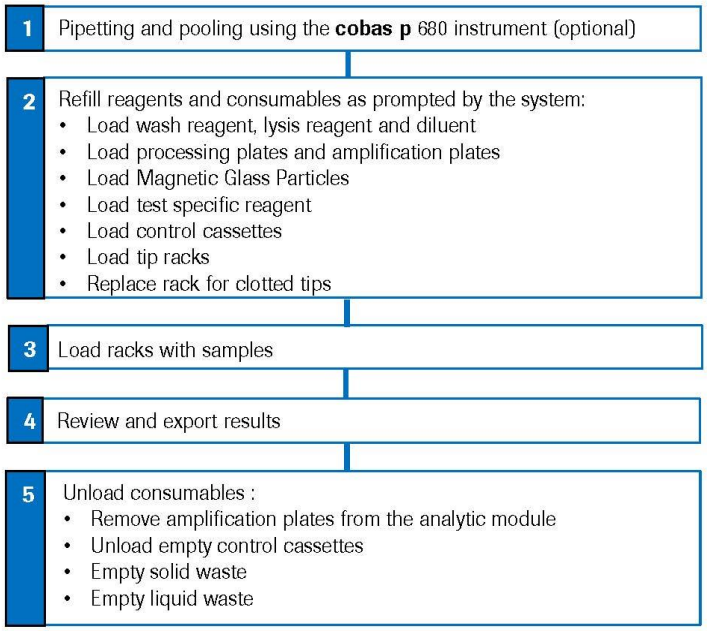
Procedural notes

- Do not use **cobas**® HEV test reagents, **cobas**® HEV 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 for proper maintenance of instruments.

Running the cobas® HEV test

The test procedure is described in detail in the **cobas**® **6800/8800** Systems Operator's Manual and the **cobas p** 680 instrument Operator's Manual. Figure 2 below summarizes the procedure.

Figure 2 **cobas**® HEV test procedure



Results

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

Quality control and validity of results

- One negative control [(-) C] and one positive control [HEV (+) C] is 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**
HEV (+) C	Q02	Invalid	The entire batch is assigned invalid if the result for the HEV (+) C is invalid.

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

Interpretation of results

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

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

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

Table 10 Target results for individual target result interpretation

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

Repeat testing of individual sample(s)

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

Procedural limitations

- The **cobas**® HEV test has been evaluated only for use in combination with the **cobas**® HEV 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 HEV RNA is dependent on the number of virus particles present in the sample and may be affected by sample collection, storage and handling, patient factors (i.e., age, presence of symptoms), and/or stage of infection and pool size.
- Though rare, mutations within the highly conserved regions of a viral genome covered by the **cobas**® HEV test, may affect primers and/or probe binding resulting in the failure to detect presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.

Non-clinical performance evaluation

Key performance characteristics

Living donor samples

Limit of Detection (LoD)

WHO International Standard

The limit of detection (LoD) of the **cobas**® HEV test for HEV RNA was determined using the WHO International Standard for HEV (PEI code 6329/10).

For the WHO International standard, 3 independent dilution series of the viral standard were prepared with normal, virus-negative (HEV) human EDTA-plasma. Each dilution series was tested using 3 different lots of the **cobas**® HEVtest kits with approximately 63 replicates per lot, for a total of approximately 189 replicates per concentration. For HEV 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 HEV 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
HEV	IU/mL	18.6	15.9	22.6

Table 12 Reactivity rates summary for HEV in EDTA plasma

HEV RNA concentration (IU/mL)	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
40	187	187	100.0%	98.4%
20	179	188	95.2%	91.8%
10	165	189	87.3%	82.6%
6	113	187	60.4%	54.2%
2	52	189	27.5%	22.2%

Reproducibility

The reproducibility of the **cobas**® HEV test on the **cobas**® 6800/8800 Systems was determined using the WHO International Standard for HEV (PEI code 6329/10). This study consisted of testing 3 panels of HEV at concentrations of approximately 0.5 x, 1 x and 2 x the LoD of the **cobas**® HEV test. 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 the **cobas**® HEV test
- instrument-to-instrument variability using 3 different **cobas**® 8800 Systems

Approximately 21 replicates were tested with each of the 3 panels for 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 HEV tested across 3 days, 3 reagent lots, and 3 **cobas**® 8800 Systems. The **cobas**® HEV test is reproducible over multiple days, reagent lots and multiple instruments. The results from reagent lot-to-lot variability are summarized in Table 13.

Table 13 cobas® HEV 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
HEV	2 x LoD	1	100.0% (61/61)	94.1%	100.0%
		2	100.0% (63/63)	94.3%	100.0%
		3	100.0% (63/63)	94.3%	100.0%
	1 x LoD	1	88.9% (56/63)	78.4%	95.4%
		2	96.8% (60/62)	88.8%	99.6%
		3	100.0% (63/63)	94.3%	100.0%
	0.5 x LoD	1	82.5% (52/63)	70.9%	90.9%
		2	95.2% (60/63)	86.7%	99.0%
		3	84.1% (53/63)	72.7%	92.1%

Genotype verification

The performance of the **cobas**® HEV test to detect 4 genotypes of HEV was determined by testing a total of 16 unique clinical samples and 7 HEV cultured isolates with known genotypes. All samples were quantified traceable to the HEV WHO standard. All 16 clinical samples were tested after dilution with normal, virus-negative (HEV) human EDTA-plasma to 5 x LoD of the **cobas**® HEV test, of which 10 samples were also tested neat. All 7 cultured isolates were tested after dilution with normal, virus-negative (HEV) human EDTA-plasma to 5 x LoD of the **cobas**® HEV test. All clinical samples and cultured isolates were detected at neat and/or at 5 x LoD (Table 14).

Table 14 HEV clinical samples and cultured isolates

Genotype	Clinical samples		Cultured isolates
	% Reactive (reactive/samples tested) neat	% Reactive (reactive/samples tested) diluted to 5 x LoD	% Reactive (reactive/samples tested) diluted to 5 x LoD
1	Not tested*	Not tested*	100.0% (3/3)
2	Not tested*	Not tested*	100.0% (1/1)
3	100.0% (10/10)	100.0% (10/10)	Not tested*
4	Not tested*	100.0% (6/6)	100.0% (3/3)

*Insufficient volume to test at neat/diluted

Analytical specificity

The analytical specificity of the **cobas**® HEV test was evaluated for cross-reactivity with 28 microorganisms at 10^6 particles, copies, or PFU/mL, which included 21 viral isolates, 6 bacterial strains and 1 yeast isolate (Table 15). The microorganisms were added to normal, virus-negative human EDTA-plasma and tested with and without HEV added to a concentration of approximately 3 x LoD of the **cobas**® HEV test for each virus. The tested microorganisms do not cross-react or interfere with the **cobas**® HEV test.

Table 15 Microorganisms tested for analytical specificity

Viruses	Flavivirus	Bacteria	Yeast
Adenovirus 5	West Nile Virus	*Escherichia coli*	*Candida albicans*
Cytomegalovirus	Dengue Virus type 1	*Propionibacterium acnes*	
Epstein-Barr Virus	Usutu Virus	*Staphylococcus aureus*	
Herpes Simplex Virus type 1		*Staphylococcus epidermidis*	
Herpes Simplex Virus type 2		*Streptococcus viridans*	
Hepatitis A Virus		*Staphylococcus haemolyticus*	
Hepatitis B Virus			
Hepatitis C Virus			
Hepatitis G Virus			
Human Immunodeficiency Virus (HIV-1 Group M)			
Human Immunodeficiency Virus (HIV-2)			
Human T-cell lymphotropic Virus type I			
Human T-cell lymphotropic Virus type II			
Human Herpes Virus 6			
Influenza Virus A			
Parvovirus B19			
Chikungunya Virus			
Varicella Zoster Virus			

Plasma samples from each of the disease states (Table 16) were tested without HEV and with HEVadded to a concentration of approximately 3 x LoD of the **cobas**® HEV test. These disease states do not cross-react or interfere with the **cobas**® HEV test.

Table 16 Disease states samples tested for analytical specificity

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

Analytical specificity – interfering substances

Endogenous interference substances

Plasma samples with abnormally high levels of triglycerides (up to 33.2 g/L), hemoglobin (up to 4.7 g/L), unconjugated bilirubin (up to 0.28 g/L), albumin (up to 60 g/L), and human DNA (up to 0.004 g/L) were tested with and without HEV added to a concentration of approximately 3 x LoD of the **cobas**® HEV test. Samples containing these endogenous substances did not interfere with the sensitivity or specificity of **cobas**® HEV test.

Exogenous interference substances

Normal, virus-negative (HEV) human EDTA-plasma samples containing abnormally high concentrations of drugs (Table 17) were tested with and without HEV added to a concentration of 3 x LoD of the **cobas**® HEV test. These exogenous substances did not interfere with the sensitivity or specificity of the **cobas**® HEV test.

Table 17 Clinical samples tested with drugs

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

Correlation

Performance evaluation of the cobas® HEV test compared to the Realstar HEV RT-PCR Kit 1.0 test

The performance of the **cobas**® HEV test and the Realstar® HEV RT-PCR Kit 1.0 test (Altona Diagnostics) was compared using 100 individual HEV NAT-positive plasma samples. 100 positive samples were tested neat and 67 positive samples were tested diluted 1:6. In addition, 100 HEV negative plasma samples were tested neat with both methods.

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

For positive samples, both methods were in agreement based on the McNemars's test indicating that the performance of **cobas**® HEV test and Realstar® HEV RT-PCR Kit 1.0 test are equivalent (Table 18).

Table 18 Correlation of positive samples (neat)

Methods		HEV results	
Realstar® HEV RT-PCR Kit 1.0 test	**cobas® HEV**	**Neat**	**Diluted 1:6**
Non-reactive	Non-reactive	0	3
Reactive	Non-Reactive	0	3
Non-reactive	Reactive	1	9
Reactive	Reactive	99	52
Total		100	67
McNemar's Test, p-value (two-sided, α=0.05)		1.00	0.09

Whole System Failure

The Whole System Failure rate for the **cobas**® HEV test was determined by testing 100 replicates of EDTA plasma spiked with HEV. These samples were tested at a target concentration of approximately 3 x LoD and were run in pools of 1 (undiluted). The study was performed using the **cobas**® 8800 System with **cobas p** 680 instrument (pipetting and pooling).

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

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.

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
	For IVD Performance Evaluation Only		
	This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.		

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

Roche Diagnostics (Schweiz) AG
Industriestrasse 7
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Roche Diagnostics GmbH
Sandhofer Straße 116
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Roche Diagnostics, SL
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Barcelona, Spain

Roche Diagnostica Brasil Ltda.
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05321-010 São Paulo, SP Brazil

Roche Diagnostics
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(For Technical Assistance call:
Pour toute assistance technique,
appeler le: 1-877 273-3433)

Roche Diagnostics
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38240 Meylan, France

Distributore in Italia:
Roche Diagnostics S.p.A.
Viale G. B. Stucchi 110
20052 Monza, Milano, Italy

Distribuidor em Portugal:
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Estrada Nacional, 249-1
2720-413 Amadora, Portugal

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