Rx Only

cobas® Factor II and Factor V Test

For in vitro diagnostic use

IVD

cobas® Factor II and Factor V Test	96 Tests	P/N: 07948352190

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Intended use

The **cobas**® Factor II and Factor V Test is an *in vitro* diagnostic device that uses real-time Polymerase Chain Reaction (PCR) for the detection and genotyping of the human Factor II (Prothrombin) G20210A mutation and the human Factor V Leiden G1691A mutation, from genomic DNA obtained from K_2EDTA whole blood specimens, as an aid in diagnosis of patients with suspected thrombophilia. The **cobas**® Factor II and Factor V Test and the **cobas z** 480 analyzer are used together for automated amplification and detection.

Summary and explanation of the test

Background

Thrombophilia is a condition with a predisposition to develop thrombosis (e.g., blood clots) due to either an inherited or acquired defect in the coagulation system. Blood clots may form in either the venous or arterial vascular system and can lead to Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE). Collectively, DVT and PE are known as Venous Thromboembolism (VTE).[1] VTE is the third most common cause of cardiovascular death after acute coronary syndrome and stroke.[2]

Inherited thrombophilia is most frequently caused by Factor V or Factor II (Prothrombin) gene mutation. The Factor V Leiden mutation is a single point mutation (G to A at position 1691, or G1691A) of the human Factor V gene that results in substitution of arginine to glutamine at position 506 (R506Q) in the Factor V protein. Factor V Leiden mutation renders the protein partially resistant to inactivation by activated protein C (APC). APC resistance is regarded as the most prevalent coagulation abnormality associated with VTE.[3,4] Genetic analysis has demonstrated that Factor V Leiden mutation, which has a relatively high prevalence in the general population (e.g. about 5% in Caucasians), may account for 85% to 95% of APC resistance cases.[4] In addition to the Factor V G1691A mutation, molecular genetic testing for Factor II G20210A (G to A at position 20210) is recommended as this mutation is present in 1-3% of the general population and its involvement in VTE is well established.[5-7] Evaluation of a patient's risk for hereditary thrombophilia through a Factor II and Factor V genotyping test is critical for diagnosis and clinical management of patients with thrombophilia.

Principles of the procedure

The **cobas**® Factor II and Factor V Test uses real-time PCR analysis of genomic DNA samples isolated from K_2EDTA whole blood to determine the genotype of the Factor II gene at position 20210 and/or the genotype of the Factor V gene at position 1691. The test is performed on the **cobas z** 480 analyzer. A positive control (**F2F5 PC**) and negative control (**F2F5 NC**) are included in each run to confirm the validity of the run.

Sample preparation

Isolation of genomic DNA from K_2EDTA whole blood specimens may be achieved using DNA isolation methods that yield DNA of sufficient concentration ($\geq$ 0.1 ng/µL).

PCR amplification

Target selection

The **cobas**® Factor II and Factor V Test contains two PCR primer pairs that amplify a 173 base pair region of the Factor II gene and a 233 base pair region of the Factor V gene that contain the sites of the Factor II (prothrombin) and Factor V Leiden mutations, respectively. Both wild type and mutant alleles are amplified for each gene.

Target amplification

Thermus species Z05 DNA polymerase and PCR primers are used for amplification of the Factor II and Factor V target regions. First, the PCR mixture is heated to denature the genomic DNA and expose the primer target sequences. As the mixture cools, the upstream and downstream primers anneal to the target DNA sequences in the Factor II and Factor V genes, and Z05 DNA polymerase, in the presence of divalent metal cation and excess deoxynucleotide triphosphates (dNTPs), extends each annealed primer using the target DNA as a template. This completes the first cycle of PCR, yielding double-stranded DNA copies (amplicons) of the Factor II target region and the Factor V target region. This process is repeated for a number of cycles, with each cycle effectively doubling the amount of Factor II and Factor V DNA for the target regions.

Automated real-time genotyping

The **cobas**® Factor II and Factor V Test utilizes real-time PCR technology to determine the genotype of the Factor II gene at position 20210 and the genotype of the Factor V gene at position 1691. The reaction contains four oligonucleotide probes, two for Factor II (G20210A mutation and wild type sequences), and two for Factor V (G1691A mutation and wild type sequences). Each oligonucleotide probe is labeled with a fluorescent dye that serves as a reporter, and with a quencher molecule that absorbs (quenches) fluorescent emissions from the reporter dye within an intact probe. During each cycle of amplification, the probes bind to the complementary regions of amplicon DNA, and are cleaved by the 5' to 3' nuclease activity of Z05 DNA Polymerase. The wild type and mutant probes are cleaved only when they are bound to the corresponding wild type or mutant Factor II and Factor V sequences, respectively.

Cleavage of probe molecules allows the reporter dye to separate from the quencher, so that the fluorescence of the reporter dye can be measured when the reaction is irradiated with the appropriate wavelength of light. The Factor II and Factor V wild type and mutant probes are labeled with different reporter dyes. Detection of elevated fluorescence for any of the reporter dyes during real-time PCR indicates that the Factor II or Factor V allele corresponding to that dye is present in the reaction. The Factor II and Factor V genotypes are determined based on which alleles are detected for each gene. If both mutant and wild type alleles are detected for a gene, the genotype is heterozygous. If only the wild type or mutant alleles are detected, then the genotype is wild type or homozygous mutant, respectively. If neither the wild type nor the mutant allele is detected for either Factor II or Factor V, the result for both genes is invalid.

Selective amplification

Selective amplification of target nucleic acid from the sample is achieved in the **cobas**® Factor II and Factor V Test by the use of AmpErase (uracil-N-glycosylase enzyme) and deoxyuridine triphosphate (dUTP).[8] The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine but not DNA containing thymidine. Deoxyuridine is not present in naturally occurring DNA but is always present in amplicon DNA due to the use of dUTP in place of deoxythymidine triphosphate as one of the deoxynucleotide triphosphates in the Master Mix reagent; therefore, only amplicon DNA contains deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by AmpErase enzyme prior to amplification of the target DNA. The AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step at alkaline pH, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. The AmpErase enzyme is inactive at temperatures above 55°C, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon.

Materials and reagents

Materials and reagents provided

Kit	Reagents and Ingredients	Quantity per Test	Safety Symbol and Warning[a]
cobas® Factor II and Factor V Test 96 Tests (P/N: 07948352190)	**F2F5 MMX** (Factor II and Factor V Master Mix) Thermostabile DNA polymerase, AmpErase (uracil-N-glycosylase), oligonucleotide PCR primers, fluorescent labeled oligonucleotide probes, deoxynucleotide triphosphates, glycerol, dimethyl sulfoxide, non-ionic detergent and 0.09% Sodium azide	4 x 0.6 mL	N/A
	F2F5 COFACTOR (Factor II and Factor V Cofactor) Manganese acetate, magnesium acetate, bovine serum albumin, and 0.09% Sodium azide	4 x 0.15 mL	N/A - Not a hazardous substance or mixture. EUH210 Safety data sheet available on request.
	F2F5 PC (Factor II and Factor V Positive Control) Non-infectious nucleic acids in a buffered solution containing 0.05% Sodium azide	4 x 0.05 mL	N/A
	F2F5 NC (Factor II and Factor V Negative Control) Buffered solution containing 0.05% Sodium azide	4 x 0.4 mL	N/A

[a]Product safety labeling primarily follows EU GHS guidance.

Reagent storage and handling

Kit	Storage Temperature	Storage Time
cobas® Factor II and Factor V Test	2°C to 8°C	Once any kit reagent is opened it is stable for up to two uses over 90 days, or until the expiration date, whichever is earlier.

Note: Do not freeze reagents.

Additional materials required

Materials	P/N
Bleach	Any vendor
70% Ethanol	Any vendor
cobas® 4800 System Microwell Plate (AD-Plate) and sealing film	Roche 05232724001
cobas® 4800 System sealing film applicator (supplied with the installation of the **cobas**® 4800 System)	Roche 04900383001
Adjustable pipettors* (Capable of pipetting 5 – 1000 µL)	Any vendor
Aerosol barrier or positive displacement DNase-free pipette tips	Any vendor
Bench top microcentrifuge* (capable of 20,000 x g)	Eppendorf 5430 or 5430R or equivalent
Microcentrifuge tubes (1.5-mL RNase/DNase free/ PCR grade)	Life Technologies AM12400 or Eppendorf 022364120 or equivalent
Conical and microcentrifuge tube racks	Any vendor
Vortex mixer*	Any vendor
Disposable powder-free gloves	Any vendor

*All equipment should be properly maintained according to manufacturer's instructions.

For more information regarding the materials sold separately, contact your local Roche representative.

Instrumentation and software required but not provided

Required Instrumentation and Software, Not Provided
cobas z 480 analyzer
cobas® 4800 System Control Unit with System Software version 2.2 or higher
Factor II and Factor V Analysis Package Software version 1.0 or higher
Barcode Reader ext USB
Printer (e.g., HP P2055d)

For more information regarding the materials sold separately, contact your local Roche representative.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay.

- For *in vitro* diagnostic use only
- Safety Data Sheets (SDS) are available upon request from your local Roche office.
- All samples should be handled as if infectious using good laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories[9] and in the CLSI Document M29-A4.[10]
- The use of sterile disposable pipettes and DNase-free pipette tips is recommended.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas.
- Wash hands thoroughly after handling samples and kit reagents.
- Wear eye protection, laboratory coats and disposable gloves when handling any reagents. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills occur, dilute with water before wiping dry.
- 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.

Note: Commercial liquid household bleach typically contains sodium hypochlorite at a concentration of 5.25%. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.

Contamination

- Gloves must be worn and must be changed between handling samples and the **cobas**® Factor II and Factor V Test reagents to prevent contamination. Avoid contaminating gloves when handling samples.
- Gloves must be changed before leaving DNA isolation areas or if contact with solutions or a sample is suspected.
- Avoid microbial contamination of reagents.
- The amplification and detection work area should be thoroughly cleaned before working MMX preparation. Supplies and equipment should be dedicated to each activity and not used for other activities or moved between areas. For example, pipettors and supplies used for DNA isolation must not be used to prepare reagents for Amplification and Detection.
- It is highly recommended that workflow in the laboratory proceed in a uni-directional manner, completing one activity before proceeding to the next activity. For example, DNA isolation should be completed before starting amplification and detection. DNA isolation should be performed in an area separate from amplification and detection. To avoid contamination of the working master mix with DNA samples, the amplification and detection work area should be thoroughly cleaned before working master mix preparation.

Integrity

- Do not use kits after their expiration dates.
- Do not pool reagents from different kits or lots.
- Do not combine reagent vials from different kit lots.
- Do not use disposable items beyond their expiration date.
- All disposable items are for one time use. Do not reuse.
- All equipment should be properly maintained according to the manufacturer's instructions.

Disposal

- **F2F5 MMX, F2F5 COFACTOR, F2F5 PC** and **F2F5 NC** contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide containing solutions down laboratory sinks, flush the drains with a large volume of cold water to prevent azide buildup.
- Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.

Spillage and cleaning

- If a spill occurs with potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 0.5% sodium hypochlorite.
- If spills occur on the **cobas**® 4800 instrument, follow the instructions in the **cobas**® **4800 System - User Assistance** to clean.
- Do not use sodium hypochlorite solution (bleach) for cleaning the **cobas z** 480 analyzer. Clean the **cobas z** 480 analyzer according to procedures described in the **cobas**® **4800 System - User Assistance**.
- For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas z** 480 analyzer, consult the **cobas**® **4800 System - User Assistance**.

Sample collection, transport, and storage

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

Sample collection and handling

The **cobas**® Factor II and Factor V Test has been developed for use on genomic DNA isolated from human whole blood samples anti-coagulated with K_2EDTA. DNA isolated from whole blood samples may be tested immediately or stored according to the recommendations of the DNA isolation kit used.

Sample transport, storage and stability

Whole blood samples anti-coagulated with K_2EDTA may be transported at 2°C to 30°C or frozen (Table 1). **cobas**® Factor II and Factor V Test results remained the same on whole blood samples when frozen and thawed up to three times. Transportation of whole blood samples must comply with country, federal, state, and local regulations for the transport of etiologic agents.[11]

Table 1 Stability of whole blood

Whole Blood Storage Temperature	15°C to 30°C	2°C to 8°C	-15°C to -25°C
Storage Time	Up to 3 days	Up to 7 days	Up to 6 weeks

Processed sample storage and stability

Genomic DNA isolated from whole blood samples anti-coagulated with K_2EDTA may be tested immediately or stored according to the recommendations of the DNA isolation kit manufacturer. Isolated DNA should be used within the recommended storage periods or before the expiration date of the commercial DNA isolation kit used to extract the DNA, whichever is earlier. In-house testing supports genomic DNA stability as shown in Table 2. Prior to using stored genomic DNA samples, pulse vortex the tube containing the sample. **cobas**® Factor II and Factor V Test results remained the same on genomic DNA samples when frozen and thawed up to three times.

Table 2 Stability of genomic DNA

Extracted DNA Storage Temperature	2°C to 8°C*	-15°C to -25°C*
Storage Time	Up to 7 days	Up to 6 weeks

*Data generated using three commercially available DNA isolation methods

Test procedure

Running the test

Table 3 cobas® Factor II and Factor V Test workflow

1	If DNA has not been isolated from the whole blood samples, perform DNA isolation
2	Remove DNA samples and test reagents from storage
3	Start the **cobas**® 4800 System
4	Perform instrument maintenance
5	Create work order and print plate layout
6	Prepare amplification reagents
7	Load microwell plate with amplification reagents
8	Load microwell plate with samples and controls
9	Seal microwell plate
10	Load microwell plate on the **cobas z** 480 analyzer
11	Start the run
12	Review results
13	With LIS: send results to LIS
14	Unload analyzer

Instructions for use

Note: Refer to the cobas® 4800 System - User Assistance for detailed operating instructions for the cobas z 480 analyzer.

Run size

A single run may include from one to 94 samples and two controls per 96-well Microwell plate. The **cobas**® Factor II and Factor V Test kit contains sufficient reagents for testing 96 samples in up to eight test batches per kit.

DNA isolation

1. Isolate genomic DNA from human whole blood anti-coagulated with K_2EDTA, using a DNA isolation method capable of yielding genomic DNA samples that are clear and colorless, and with sufficient concentration ($\geq$ 0.1 ng/µL).
2. **F2F5 NC** may be included in the DNA isolation procedure, as a control for contamination during DNA isolation, or may be used directly, without processing, in the **cobas**® Factor II and Factor V Test.
3. **F2F5 PC** is used in the **cobas**® Factor II and Factor V Test without DNA isolation. Do not perform DNA isolation on **F2F5 PC.**
4. Isolated genomic DNA may be tested immediately with the **cobas**® Factor II and Factor V Test or stored according to the recommendations of the DNA isolation kit manufacturer.

Amplification and detection

Note: To avoid contamination of working MMX with DNA samples, amplification and detection should be performed in an area separated from DNA isolation. The amplification and detection work area should be thoroughly cleaned before preparation of working MMX. For proper cleaning, all surfaces including racks and pipettors should be thoroughly wiped with 0.5% sodium hypochlorite solution followed by wiping with a 70% ethanol solution. Commercial liquid household bleach typically contains sodium hypochlorite at a concentration of 5.25%. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.

Work order and plate layout

For detailed instructions on these workflow steps, refer to the **cobas® 4800 System - User Assistance**.

Generate a work order and plate layout with the positions of all the samples and controls in the run. **F2F5 PC** is loaded into position A01 on the plate, and **F2F5 NC** is loaded into position B01 on the plate. Genomic DNA samples are then added starting with well C01 to H01, A02 to H02, as shown in Table 4.

Table 4 Plate layout for the cobas® Factor II and Factor V Test

Row / Column	01	02	03	04	05	06	07	08	09	10	11	12
A	PC[a]	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
B	NC[b]	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
C	S1[c]	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
D	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
E	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
F	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
G	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
H	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94

[a] PC = **F2F5 PC**
[b] NC = **F2F5 NC**
[c] S = Sample

Instrument set-up

1. Turn on the **cobas z** 480 analyzer. The instrument will take several minutes to warm up before the run can begin.
2. Turn on the control unit. The control unit logs into Windows automatically.
3. Double click on the **cobas**® 4800 software icon and log on to perform the run using the specified lab user ID and password.
4. Click on the "New Run" icon from the menu.
5. The "Select Test" pop up window appears. Select "PCR Only" Workflow type, then select "F2F5" and click the "OK" button.
6. When the "Work Place" Screen appears, click in the "Microwell Plate ID" field and type or scan in the Microwell Plate (MWP) Barcode. Enter comments in the "Comments" field if desired.
7. Click in the "F2F5 Test Kit-ID" field in the first kit barcode row, and type or scan the **cobas**® Factor II and Factor V Test kit ID barcode. In the "Specimen" field on the same row, enter the number of samples to be tested in the run.
    a. If a single kit lot is used, up to 94 samples may be entered in the first kit barcode row (2 results are reserved for the **F2F5 PC** and **F2F5 NC**).
    b. If multiple kits from the same lot are used (up to 4 kits), enter the **cobas**® Factor II and Factor V Test kit ID barcode in the "F2F5 Test Kit-ID" field and the number of samples to be tested with that kit in the "Specimen" field, using one kit barcode row for each kit.
    c. A maximum of 94 samples and 2 controls can be run per AD plate.

8. The "Sample ID" is automatically populated in for the control positions (A01 and B01). Type or scan in the "Sample ID" for every sample into the Sample ID column. Every Sample ID in the same run must be unique. The "Kit" and "Sample Type" fields are automatically populated.
9. For each sample, select the "Requested Result": "F2" for Factor II only, "F5" for Factor V only or "F2F5" for both Factor II and Factor V.
10. Enter comments for each sample, if desired.
11. When all information has been entered, click the "Save" button located at the bottom right-hand corner of the screen.
12. Save the file with the default file name assigned by the software.
13. Print a plate layout with the positions of all the samples and controls in the run by clicking the Print button and selecting File -> Print in the Preview window. Always fill the plate by columns, starting with the **F2F5 PC** in position A01, and the **F2F5 NC** in position B01. Map the samples beginning with position C01 and continuing down to H01, then continue with positions A02 to H02, A03 to H03, and so on, until all samples have been mapped.

Reaction set-up

Preparation of working master mix

Note: F2F5 MMX and working MMX (MMX and COFACTOR) are light-sensitive and must be protected from prolonged exposure to light. Working MMX must be stored at 2°C to 8°C in the dark.

Note: The isolated DNA samples and controls must be added within 1 hour of preparation of the working MMX. Amplification must be started within 1 hour from the time that the processed samples and controls are added to the working MMX.

Note: The F2F5 MMX may appear light blue/purple. This does not affect the performance of the reagent.

1. Calculate the volume of **F2F5 MMX** required using the following formula:

   Volume of **F2F5 MMX** required = (Number of Samples + 2 Controls +1) x 20 µL
2. Calculate the volume of **F2F5 COFACTOR** required using the following formula:

   Volume of **F2F5 COFACTOR** required = (Number of Samples + 2 Controls +1) x 5 µL

Table 5 may be used to determine the volume of **F2F5 MMX** and **F2F5 COFACTOR** needed for the preparation of working MMX based on the number of samples included in the run.

Table 5 Reagent volumes for working MMX

		Number of Samples[a]						
		1	2	3	6	9	12	24
MMX	20 µL	80	100	120	180	240	300	540
COFACTOR	5 µL	20	25	30	45	60	75	135
Total Volume of Working MMX (µL)		100	125	150	225	300	375	675

[a] MMX and COFACTOR volumes based on the number of samples to be tested + 2 controls + 1 extra reaction

3. Remove the appropriate number of **F2F5 MMX** and **F2F5 COFACTOR** vials from 2°C to 8°C storage. Vortex each reagent for 5 seconds and collect the liquid at the bottom of the tube before use.
4. Label a sterile microcentrifuge tube for working MMX for each set of up to 24 samples.
5. Add the calculated volume of **F2F5 MMX** to the labeled tube.
6. Add the calculated volume of **F2F5 COFACTOR** to the labeled tube.

7. Vortex the working MMX tube for 3 to 5 seconds to ensure adequate mixing.

Note: Samples and controls should be added to the microwell plate (AD-plate) within 1 hour after the preparation of the working MMX.

Note: Use only cobas® 4800 System Microwell Plate (AD-Plate) and Sealing film.

Preparation of plate

Note: If using stored genomic DNA samples, follow the instructions in the Processed Sample Storage and Stability section.

1. Pipette 25 µL of working **MMX** into each reaction well of the microwell plate (AD-plate) to be used in the run, according to the plate map generated previously. Do not allow the pipette tip to touch the plate outside the well.
2. Pipette 25 µL of **F2F5 PC** into well **A01** of the microwell plate (AD-plate); mix well using the pipette to aspirate and dispense within the well a minimum of two times.
3. Using a new pipette tip, pipette 25 µL of **F2F5 NC** into well **B01** of the microwell plate (AD-plate); mix well using pipette to aspirate and dispense within the well a minimum of two times.

Note: Each run must contain F2F5 PC in well A01 and F2F5 NC in well B01, or the run will be invalidated by the cobas z480 analyzer.

Note: Change gloves as needed to protect against sample-to-sample contamination and external PCR reaction tube contamination.

4. Using a new pipette tip for each sample, add 25 µL of each DNA sample to the appropriate well of the microwell plate (AD-plate), according to the plate layout prepared above, starting from position C01. Mix each well by using the pipettor to aspirate and dispense within the well a minimum of two times. Ensure that all liquid is collected at the bottom of the wells.

Note: Pulse vortex stored genomic DNA samples before use.

5. Cover the microwell plate (AD-plate) with sealing film (supplied with the plates). Use the sealing film applicator to seal the film firmly to the microwell plate (AD-plate).
6. Confirm that all liquid is collected at the bottom of each well before starting PCR.

Note: Amplification must be started within 1 hour from the time that the processed samples and controls are added to the working MMX.

Starting PCR

Refer to the **cobas® 4800 System - User Assistance** for detailed instructions on the workflow steps.

Results

Interpretation of results

Note: All run and sample validation is performed by the cobas® 4800 software.

Note: A valid test run may include both valid and invalid sample results.

For a valid run, sample results are interpreted as shown in Table 6.

Table 6 Result interpretation for the cobas® Factor II and Factor V Test

Test Result	Interpretation
WT F2	The genomic DNA sample is wild type for the Factor II (prothrombin) mutation. The nucleotide at position 20210 of the Factor II gene is G on both alleles.
HET F2	The genomic DNA sample is heterozygous for the Factor II (prothrombin) mutation. The nucleotide at position 20210 of the Factor II gene is G on one allele and A on one allele.
MUT F2	The genomic DNA sample is homozygous mutant for the Factor II (prothrombin) mutation. The nucleotide at position 20210 of the Factor II gene is A on both alleles.
WT F5	The genomic DNA sample is wild type for the Factor V (Leiden) mutation. The nucleotide at position 1691 of the Factor V gene is G on both alleles.
HET F5	The genomic DNA sample is heterozygous for the Factor V (Leiden) mutation. The nucleotide at position 1691 of the Factor V gene is G on one allele and A on one allele.
MUT F5	The genomic DNA sample is homozygous mutant for the Factor V (Leiden) mutation. The nucleotide at position 1691 of the Factor V gene is A on both alleles.
Invalid	Sample result is invalid. Repeat the testing of samples with invalid results following the instructions outlined in the “Retesting of Samples with Invalid Results” section below.
Failed	Failed run due to hardware or software failure. Contact your local Roche office for technical assistance.

Retesting of samples with invalid results

1. If the run is invalid, repeat the entire test procedure for all samples, starting with genomic DNA.
2. If the run is valid but individual sample results are invalid, repeat the test for the invalid sample(s), starting with the genomic DNA. If the repeat test is still invalid, repeat the genomic DNA isolation from the whole blood sample(s).

Quality control and validity of results

One set of **cobas**® Factor II and Factor V controls (**F2F5 PC** and **F2F5 NC**) are included in each run. A run is valid if both **F2F5 PC** and **F2F5 NC** are valid. If **F2F5 PC** or **F2F5 NC** are invalid, the entire run is invalid and must be repeated. If the **F2F5 PC** or **F2F5 NC** are consistently invalid, contact your local Roche office for technical assistance.

List of result flags

Result flags may be found under the Results tab. The source of a flag is indicated in the flag code as outlined in the following Table 7. Table 8 lists all result interpretation flags that are relevant for the user.

Table 7 Flag source

Flag code starts with	Flag source	Example
M*	Multiple or other reasons	M6
R	Result interpretation	R20
Z*	Analyzer	Z1

* Refer to the **cobas**® 4800 System - User Assistance.

Table 8 List of result interpretation flags

Flag Code	Severity	Description	Recommended Action
R2900, R2904, R2908, R2912	Error	Positive Control Invalid	Repeat the run. Refer to the test-specific instructions for use. These flag codes indicate that the elbow determination algorithm encountered an error which may occur in the event of an atypical or noisy fluorescence pattern.
R2901, R2905, R2909, R2913	Error	Positive Control Invalid	Repeat the run. Refer to the test-specific instructions for use. These flag codes indicate that a negative result occurred in at least one channel for the Positive Control.
R2902, R2906, R2910, R2914	Error	Positive Control Invalid	Repeat the run. Refer to the test-specific instructions for use. These flag codes indicate that an observed Ct value for the Positive Control was above the established threshold (i.e. elbow too high). Possible reasons could be: 1. Incorrect preparation of the working Master Mix 2. Pipetting error when adding working Master Mix into a well of the microwell plate 3. Pipetting error when adding Positive Control into a well of the microwell plate.
R2903, R2907, R2911, R2915	Error	Positive Control Invalid	Repeat the run. Refer to the test-specific instructions for use. These flag codes indicate that an observed Ct value for the Positive Control was below the established threshold (i.e. elbow too low). This error may occur in the event of DNA contamination.
R2916	Error	Positive Control Not Detected	Repeat the run. Refer to the test-specific instructions for use. This flag code indicates that a negative result occurred for the Positive Control in all channels. Positive Control DNA may have not been added to the control well.
R2917, R2919, R2921, R2923	Error	Negative Control Invalid	Repeat the run. Refer to the test-specific instructions for use. These flag codes indicate that the elbow determination algorithm encountered an error which may occur in the event of an atypical or noisy fluorescence pattern.
R2918, R2920, R2922, R2924	Error	Negative Control Invalid	Repeat the run. Refer to the test-specific instructions for use. These flag codes indicate that a positive result occurred for the Negative Control (i.e. a contamination event occurred).
R2925, R2928, R2931, R2934, R2940, R2941, R2942, R2943	Error	Result Invalid	Repeat the run. Refer to the test-specific instructions for use. These flag codes indicate that the elbow determination algorithm encountered an error which may occur in the event of an atypical or noisy fluorescence pattern.
R2926, R2929, R2932, R2935	Error	Result Invalid	Repeat the run. Refer to the test-specific instructions for use. These flag codes indicate that an atypically high Ct value was observed for the sample.
R2927, R2930, R2933, R2936	Error	Result Invalid	Repeat the run. Refer to the test-specific instructions for use. These flag codes indicate that an atypically low Ct value was observed for the sample.
R2937, R2938	Error	Result Invalid	Repeat the sample. Refer to the test-specific instructions for use. These flag codes indicate that the results for one of the genes (i.e. Factor II or Factor V) were not valid. The absence of a valid result for both genes removes the Internal Control function each gene serves for the other and suggests: 1. Poor quality genomic DNA from the sample 2. Inadequate sample processing 3. The presence of PCR inhibitors in the sample 4. Rare mutations within the regions of the Genomic DNA covered by the primers and/or probes 5. Sample DNA may have not been added to one or more wells 6. Other factors.
R2939	Error	Targets Not Detected	Repeat the run. Refer to the test-specific instructions for use. This flag code indicates that a negative result occurred for the sample in all channels. Genomic DNA may have not been added to the well.
R2944, R2945	Error	Result Invalid	Repeat the sample. Refer to the test-specific instructions for use. These flag codes indicate that an atypical relationship between certain data analysis parameters was observed for the sample.

Procedural limitations

1. Valid results are dependent on the amount of genomic DNA present in the sample and may be affected by sample integrity, concentration of isolated DNA, and the presence of interfering substances.
2. DNA isolation methods should be capable of yielding genomic DNA that is clear and colorless, and of a concentration ≥ 0.1 ng/µL.
3. Hemoglobin is an inhibitor of PCR. Genomic DNA isolated from whole blood should appear clear and colorless. Samples that contain a red, pink or rust colored tint, color, or have any appearance other than clear and colorless may yield invalid or incorrect results, and should not be tested.
4. Reliable results are dependent on adequate transport, storage and processing. Follow the procedures in these Instructions for Use and in the **cobas**® 4800 System - User Assistance.
5. The inclusion of AmpErase enzyme into the **cobas**® Factor II and Factor V Test Master Mix enables selective amplification of target DNA; however, good laboratory practices and careful adherence to the procedures specified in these Instructions for Use are necessary to avoid contamination of reagents.
6. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 4800 System.
7. Only the **cobas z** 480 analyzer has been validated for use with this product. No other thermal cycler with real-time optical detection can be used with this product.
8. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to another, users perform method correlation studies in their laboratory to qualify technology differences.
9. Though rare, mutations within the genomic DNA regions of the Factor II gene and Factor V gene covered by the primers or probes used in the **cobas**® Factor II and Factor V Test may result in failure to detect the presence of the Factor II G20210A mutation or the Factor V G1691A mutation.
10. The presence of PCR inhibitors may cause invalid or incorrect results.
11. Commercial DNA isolation kits often contain a chaotropic salt, such as guanidine hydrochloride, in the lysis reagent, and ethanol in the wash buffer. Chaotropic salts or ethanol in genomic DNA samples may cause invalid or incorrect results. Follow the manufacturer's instructions for DNA isolation methods, to ensure that lysis reagents and wash buffers are properly removed during the DNA isolation process.

Non-clinical performance evaluation

The following data are intended to demonstrate the analytical performance of the **cobas**® Factor II and Factor V Test.

Analytical sensitivity

To determine the minimum input of genomic DNA necessary to yield correct Factor II and Factor V genotype results, genomic DNA was isolated from three K_2EDTA whole blood samples (Factor II heterozygous, Factor V heterozygous, Factor V homozygous mutant) and one cell line (Factor II homozygous mutant), using three different commercial DNA isolation methods for the whole blood samples, and one method for the cell line. Each genomic DNA sample was tested with the **cobas**® Factor II and Factor V Test at 10 concentrations: undiluted (which varied between 6 and 38 ng/µL) and nine serial dilutions from 1.0 to 0.0001 ng/µL. Each dilution was tested with 24 replicates with each of two kit lots, for a total of 48 replicates per concentration per genomic DNA sample. The undiluted genomic DNA samples were tested with six replicates with each kit lot, for a total of 12 replicates per genomic DNA sample.

The rate of correct Factor II and Factor V genotype results across all four samples, all three DNA isolation methods and both kit lots was 98% at 0.01 ng/µL and 100% at all higher concentrations (Table 9). The **cobas**® Factor II and Factor V Test is designed to yield Invalid results if the input DNA concentration is too low. There were no incorrect genotype results in the study. The limit of detection is 0.01 ng/µL, which is 10 times lower than the lowest recommended DNA input concentration.

Table 9 Analytical sensitivity of the cobas® Factor II and Factor V Test

Concentration (ng/µL)	Number	cobas® Factor II and Factor V Test Results		
		Number (%) Correct	Number (%) Incorrect	Number (%) Invalid
Undiluted*	120	120 (100%)	0 (0%)	0 (0%)
1	480	480 (100%)	0 (0%)	0 (0%)
0.3	480	480 (100%)	0 (0%)	0 (0%)
0.1	480	480 (100%)	0 (0%)	0 (0%)
0.03	480	480 (100%)	0 (0%)	0 (0%)
0.01	480	473 (98%)	0 (0%)	7 (2%)
0.003	480	86 (18%)	0 (0%)	394 (82%)
0.001	480	0 (0%)	0 (0%)	480 (100%)
0.0003	480	0 (0%)	0 (0%)	480 (100%)
0.0001	480	0 (0%)	0 (0%)	480 (100%)
0	480	0 (0%)	0 (0%)	480 (100%)

*6 to 38 ng/µL

Upper limit of DNA input

To evaluate higher concentrations of DNA input for the **cobas**® Factor II and Factor V Test, genomic DNA was isolated from four K_2EDTA whole blood samples using three different commercial DNA isolation methods, and concentrated genomic DNA from cell lines were added to yield total DNA concentrations of 300 ng/µL, 150 ng/µL and 75 ng/µL. Genomic DNA from Factor II heterozygous, Factor V heterozygous and Factor V homozygous mutant cell lines were added to genomic DNAs from whole blood samples of the same Factor II and Factor V genotypes. Factor II homozygous mutant cell line DNA was added to the processed eluate from a leukocyte depleted whole blood (LDWB) sample. The genomic DNA samples at 300 ng/µL, 150 ng/µL and 75 ng/µL were tested with 24 replicates with each of two kit lots, for a total of 48 replicates per concentration per genomic DNA sample. The genomic DNA samples from whole blood without added cell line DNA were tested with six replicates using each of two kit lots, for a total of 12 replicates per genomic DNA sample. All samples at 300 ng/µL, 150 ng/µL and 75 ng/µL yielded the correct Factor II and Factor V genotype results in all tests (Table 10). The LDWB samples without added cell line DNA yielded Invalid results, as expected due to the absence of leukocytes; the other genomic DNA samples from whole blood yielded correct Factor II and Factor V results. The highest recommended DNA input concentration is 150 ng/µL, half of the maximum input tested.

Table 10 Testing of higher DNA input for the cobas® Factor II and Factor V Test

Concentration (ng/µL)	Number	cobas® Factor II and Factor V Test Results		
		Number (%) Correct	Number (%) Incorrect	Number (%) Invalid
300	576	576 (100%)	0 (0%)	0 (0%)
150	576	576 (100%)	0 (0%)	0 (0%)
75	576	576 (100%)	0 (0%)	0 (0%)
Genomic DNA from neat whole blood samples (6 to 38 ng/ul)	108	108 (100%)	0 (0%)	0 (0%)
Leukocyte depleted whole blood sample[a]	36	36 (100%)	0 (0%)	0 (0%)

[a] Eluates from leukocyte-depleted whole blood sample yielded “Invalid” results due to the absence of leukocytes. These expected results are considered “correct” for the purpose of the study.

DNA isolation reproducibility

Genomic DNA was isolated from fifteen whole blood samples using three commercially available DNA isolation methods according to the manufacturer’s instructions, by two operators on three days, for a total of six DNA isolations per sample with each DNA isolation method. Each isolated genomic DNA sample was tested in triplicate with the **cobas**® Factor II and Factor V Test (Table 11). Ninety-nine and four tenths percent (99.4%) of the results with the **cobas**® Factor II and Factor V Test were in agreement with the Factor II and Factor V genotypes by bi-directional Sanger DNA sequencing. One genomic DNA sample isolated with method A was rust colored and yielded invalid results in all three tests. Isolated DNA samples should appear clear and colorless. DNA samples with any appearance other than clear and colorless should not be tested as they may yield invalid or incorrect results.

Table 11 DNA isolation reproducibility

DNA Isolation Method	Total Number of		Number (%) of							
	DNA Isolations	Tests	Correct Results			Incorrect Results		Invalid Results	
A	90	270	267[a]	(98.9%)	[96.8 – 99.8][b]	0	(0.0%)	3[a]	(1.1%)
B	90	270	268[c]	(99.3%)	[97.4 – 99.9][b]	1[c]	(0.4%)	1[c]	(0.4%)
C	90	270	270	(100.0%)	[98.6 – 100.0][b]	0	(0.0%)	0	(0.0%)
Total	270	810	805	(99.4%)	[98.6 – 99.8][b]	1	(0.1%)	4	(0.5%)

[a] One sample isolated with method A was rust-colored and generated 3 invalid results. Only clear and colorless DNA samples should be tested. DNA samples with any appearance other than clear and colorless should not be tested, as they may yield invalid or incorrect results.

[b] 95% two-sided confidence interval

[c] The triplicate results from one sample isolated with method B were inconsistent: 1 correct, 1 incorrect, 1 invalid. The sample eluate was re-tested in triplicate and all results were correct upon re-testing.

Analytical specificity

To determine the effect of known single nucleotide polymorphisms (SNPs) close to the Factor V Leiden mutation (G1691A) and the Factor II (prothrombin) mutation (G20210A), plasmid DNAs containing eight known SNPs (A20207C, C20209T, A20218G, C20221T, G1689A, C1690T, A1692C and A1696G) were tested. The Factor II SNP plasmids and the Factor V SNP plasmids were wild type at positions 20210 and 1691, respectively. Each SNP plasmid DNA was tested alone, and in combination with wild type Factor II plasmid DNA, wild type Factor V plasmid DNA, wild type Factor II and wild type Factor V plasmid DNAs, and with genomic DNA from wild type whole blood.

None of the SNP plasmids caused false positive results for the Factor II (prothrombin) or Factor V Leiden mutations. All four Factor II SNP plasmids and three of four Factor V SNP plasmids were detected as wild type Factor II or Factor V DNA, respectively. One Factor V SNP plasmid (G1689A) was not detected by the **cobas**® Factor II and Factor V Test. If this SNP is present on both alleles, the test result would be invalid.

Interfering substances

Triglycerides (37 mM), bilirubin (conjugated and unconjugated, 342 µM), and cholesterol (13 mM) did not interfere with the **cobas®** Factor II and Factor V Test when added to whole blood at concentrations recommended by CLSI.[12] Hemoglobin did not interfere with the **cobas®** Factor II and Factor V Test when spiked into whole blood to yield a total hemoglobin concentration of ~25.8 - 31 g/dL. The anticoagulant K_2EDTA did not interfere when it was tested at 5.7 mg/mL, which is approximately 3 times the concentration of K_2EDTA in whole blood when the blood collection tube is filled to capacity. Heparin, coumadin (Warfarin), rivaroxaban (Xarelto), and dabigatran etexilate (Pradaxa) did not interfere with the **cobas**® Factor II and Factor V Test.

A commercial extraction buffer containing Guanidine Hydrochloride, a common ingredient in commercial DNA extraction buffers, interfered with the **cobas®** Factor II and Factor V Test when present in genomic DNA samples at a concentration of 2.5% (v/v). Ethanol, a common ingredient in the wash buffers of commercial DNA isolation methods, interfered with the **cobas**® Factor II and Factor V Test when added to genomic DNA samples to a concentration of 5% (v/v). For both substances, observed interference caused invalid results.

Repeatability, lot-to-lot

The lot-to-lot repeatability of the **cobas**® Factor II and Factor V Test was evaluated by testing genomic DNA samples isolated from seven K_2EDTA whole blood samples with three lots of the **cobas**® Factor II and Factor V Test. The study was conducted over five non-consecutive days with two operators, two **cobas z** 480 analyzers, one run per day per kit lot, and two replicates per run, for a total of 60 replicates of each sample (Table 12). The **cobas**® Factor II and Factor V Test results

were compared to the Factor II and Factor V genotypes by bi-directional Sanger DNA sequencing. The overall agreement between **cobas®** Factor II and Factor V Test results and nucleic acid sequencing was 100% across all samples and reagent lots (one-sided, lower 95% confidence limit 99.3%).

Table 12 Lot-to-lot repeatability

Sample ID	Factor II Genotype	Factor V Genotype	Number of Tests per Lot	Number (%) Correct Genotype Results			
				Lot 1	Lot 2	Lot 3	All Lots
S1	Wild type	Wild type	20	20 (100%)	20 (100%)	20 (100%)	60 (100%)
S2	Wild type	Wild type	20	20 (100%)	20 (100%)	20 (100%)	60 (100%)
S3	Wild type	Heterozygous	20	20 (100%)	20 (100%)	20 (100%)	60 (100%)
S4	Heterozygous	Wild type	20	20 (100%)	20 (100%)	20 (100%)	60 (100%)
S5	Wild type	Homozygous mutant	20	20 (100%)	20 (100%)	20 (100%)	60 (100%)
S6	Homozygous mutant	Wild type	20	20 (100%)	20 (100%)	20 (100%)	60 (100%)
S7	Heterozygous	Heterozygous	20	20 (100%)	20 (100%)	20 (100%)	60 (100%)
Total			**140**	**140 (100%)**	**140 (100%)**	**140 (100%)**	**420 (100%)**

Clinical performance evaluation

Method correlation: comparison to Sanger sequencing

Frozen K_2EDTA whole blood and DNA samples (total N=300) representing the intended use population were tested at one site by the **cobas**® Factor II and Factor V Test. Bi-directional Sanger sequencing was performed at a different site. A commercially available, manual whole blood sample preparation method was used to extract DNA from the whole blood samples. Of the total, 284 samples were whole blood and represented frequently found genotypes for Factor II and Factor V. The majority of samples for the rare Factor II Homozygous Mutant/ Factor V Wild Type genotype were acquired as genomic DNA (gDNA) samples without identified isolation methodology. Genotypes and specimen types are presented in Table 13.

Table 13 Factor II and Factor V genotypes and specimen types included in the study

Factor II (F2)	Factor V (F5)	Specimen Type
Wild Type (WT F2)	Wild Type (WT F5)	Whole Blood
Wild Type (WT F2)	Heterozygous (HET F5)	Whole Blood
Heterozygous (HET F2)	Wild Type (WT F5)	Whole Blood
Heterozygous (HET F2)	Heterozygous (HET F5)	Whole Blood
Wild Type (WT F2)	Homozygous Mutant (MUT F5)	Whole Blood
Homozygous Mutant (MUT F2)	Wild Type (WT F5)	Whole Blood and gDNA

All 300 samples had valid results by bi-directional Sanger sequencing and the **cobas**® Factor II and Factor V Test. A test result was classified as correct for Factor II or Factor V if both the **cobas**® Factor II and Factor V Test and Sanger Sequencing detected the same genotype. A test result was classified as incorrect for Factor II or Factor V if the **cobas**® Factor II and Factor V Test and Sanger Sequencing detected a different genotype for Factor II or Factor V.

The Overall Percent Agreement (OPA) between the two tests for Factor II was 100% with a two-sided 95% lower confidence bound (Exact method) of 98.78%. Both Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were 100% with lower confidence bounds of 97.59% for PPA and 97.55% for NPA. The percent agreement for both the Heterozygous and Homozygous Mutant genotypes was 100%.

The OPA between the two tests for Factor V was 100% with a two-sided 95% lower confidence bound (Exact method) of 98.78%. Both PPA and NPA were 100% with lower confidence bounds of 97.62% for PPA and 97.52% for NPA. The percent agreement for both the Heterozygous and Homozygous Mutant genotypes was 100%.

Combined results are presented in Table 14.

Table 14 Performance of the cobas® Factor II and Factor V Test using bi-directional Sanger Sequencing as a reference for the identification of combined Factor II and Factor V results

Bi-Directional Sanger Sequencing Result	cobas® Factor II and Factor V Test Result						Total
	HET F2/ HET F5	HET F2/ WT F5	MUT F2/ WT F5	WT F2/ HET F5	WT F2/ MUT F5	WT F2/ WT F5	
HET F2 / HET F5	25	0	0	0	0	0	25
HET F2 / WT F5	0	105	0	0	0	0	105
MUT F2 / WT F5	0	0	21	0	0	0	21
WT F2 / HET F5	0	0	0	105	0	0	105
WT F2 / MUT F5	0	0	0	0	23	0	23
WT F2 / WT F5	0	0	0	0	0	21	21
Total	25	105	21	105	23	21	300

Reproducibility

Reproducibility of the **cobas**® Factor II and Factor V Test for the detection and genotyping of the Factor II G20210A and Factor V Leiden (G1691A) mutations was assessed at three sites using a nine-member panel: four unique K_2EDTA blood samples, three contrived blood samples and two extracted genomic DNA (gDNA) samples diluted to 0.2 ng/µL. Each site extracted DNA from the blood samples with one of three commercially available, manual sample preparation methods. Each site used one of three **cobas**® Factor II and Factor V Test lots and one instrument, with two operators per site, one run per day per operator over a period of 5 non-consecutive days.

A correct result was defined as agreement between the **cobas**® Factor II and Factor V Test result and the panel member genotype determined by Sanger sequencing; an incorrect result was defined as disagreement between the cobas test result and the panel member genotype determined by Sanger sequencing. The **cobas**® Factor II and Factor V Test results were reported in each sample for both Factor II and Factor V, and they were either valid or invalid for both mutations.

A total of 540 tests were performed across 30 valid runs: 240 were from K_2EDTA blood samples, 180 were from contrived blood samples and 120 results were from gDNA. Out of 540 results, a single invalid result from a contrived blood sample was observed. All valid Factor II and Factor V results were correct, leading to 100% agreement at all levels: genotype, site/DNA isolation method/reagent lot, sample type, operators and days. The overall invalid rate was 0.19% (1/540). Study results are summarized in Table 15 and Table 16.

Table 15 Summary of reproducibility study – Factor II

Genotype by Sequencing[a]	Correct Results/ Number of Samples Tested			Incorrect Results	Invalid Results[c]	Correct Results / Number of Valid Results (%)	95% LCB[d]
Factor II	Site1[b]/ Lot 1 Method A	Site 2[b]/Lot 2 Method B	Site 3[b]/ Lot 3 Method C				
WT[e]	100/100	100/100	100/100	0	0	300/300 (100%)	98.78%
HET[e]	60/60	59/60	60/60	0	1	179/179 (100%)	97.96%
MUT[f]	20/20	20/20	20/20	0	0	60/60 (100%)	94.04%

[a] WT: Wild Type; HET: Heterozygous; MUT: Homozygous Mutant

[b] Each site used a different DNA isolation method (A,B,C) and different **cobas**® Factor II and Factor V Test lot.

[c] Invalid results were not re-tested.

[d] Two-sided 95% Lower Confidence Bound (LCB) was calculated by the Exact method.

[e] At each site, 20 results were from gDNA samples and 20 from contrived blood samples.

[f] From contrived blood samples only

Table 16 Summary of reproducibility study – Factor V

Genotype by Sequencing[a]	Correct Results/ Number of Samples Tested			Incorrect Results	Invalid Results[c]	Correct Results / Number of Valid Results (%)	95% LCB[d]
Factor V	Site1[b]/Lot 1 Method A	Site 2[b]/ Lot 2 Method B	Site 3[b]/Lot 3 Method C				
WT[e]	100/100	100/100	100/100	0	0	300/300 (100%)	98.78%
HET[e]	60/60	59/60	60/60	0	1	179/179 (100%)	97.96%
MUT[f]	20/20	20/20	20/20	0	0	60/60 (100%)	94.04%

[a] WT: Wild Type; HET: Heterozygous; MUT: Homozygous Mutant

[b] Each site used different DNA isolation method (A,B,C) and different **cobas**® Factor II and Factor V Test lot.

[c] Invalid results were not re-tested.

[d] Two-sided 95% Lower Confidence Bound (LCB) was calculated by the Exact method.

[e] At each site, 20 results were from gDNA samples and 20 from contrived blood samples.

[f] From contrived blood samples only

Additional information

Symbols

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

Table 17 Symbols used in labeling for Roche PCR diagnostic products

	Ancillary Software		*In Vitro* Diagnostic Medical Device
	Authorized Representative in the European community		Lower Limit of Assigned Range
	Barcode Data Sheet		Manufacturer
	Batch code		Store in the dark
	Biological Risks		Contains sufficient for <*n*> tests
	Catalogue number		Temperature Limit
	Consult instructions for use		Test Definition File
	Contents of kit		Upper Limit of Assigned Range
	Distributed by		Use-by date
	For IVD Performance Evaluation Only		Global Trade Item Number
	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 18 Manufacturer and distributors

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

Roche Diagnostics (Schweiz) AG
Industriestrasse 7
6343 Rotkreuz, Switzerland

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Roche Diagnostics, SL
Avda. Generalitat, 171-173
E-08174 Sant Cugat del Vallès
Barcelona, Spain

Roche Diagnostica Brasil Ltda.
Av. Engenheiro Billings, 1729
Jaguaré, Building 10
05321-010 São Paulo, SP Brazil

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

Roche Diagnostics
201, boulevard Armand-Frappier
H7V 4A2 Laval, Québec, Canada
Pour toute assistance technique,
appeler le: 1-877-273-3433)

Roche Diagnostics
2, Avenue du Vercors
38240 Meylan, France

Distributore in Italia:
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Viale G. B. Stucchi 110
20052 Monza, Milano, Italy

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2720-413 Amadora, Portugal

Trademarks and patents

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

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