Geno-Sen's

DENGUE 1-4

Real Time PCR Kit

Quantitative



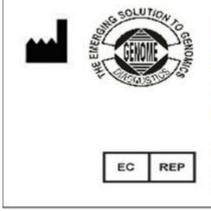
PACK INSERT

Revised Sept 2016

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Genome Diagnostics Pvt. Ltd.

(An ISO 13485:2012, 9001:2008 Certified Company)



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Dengue 1-4 Geno-Sen's Real Time PCR Kit

Contents	REF 9111022	REF 9111023	REF 9111024
			25 rxns
Dengue 1-4 Super	25 rxns x 4 Vials	25 rxns x 2 Vials	25 rxns x 1 Vials
mix.			
Mg Sol RT.	1 Vial	1 Vial	1 Vial
Dengue 1-4	1 Vial of 300µl	1 Vial of 300µl	1 Vial of 300µl
Standard 1			
1 X 10⁵ copies/µl			
Dengue 1-4	1 Vial of 300µl	1 Vial of 300µl	1 Vial of 300µl
Standard 2	-	-	-
1 X 10⁴ copies/µl			
Dengue 1-4	1 Vial of 300µl	1 Vial of 300µl	1 Vial of 300µl
Standard 3	-	-	-
1 X 10 ³ copies/μl			
Dengue 1-4	1 Vial of 300µl	1 Vial of 300µl	1 Vial of 300µl
Standard 4			•
1 X 10 ² copies/μl			
Dengue 1-4	1 Vial of 300µl	1 Vial of 300µl	1 Vial of 300µl
Standard 5			•
1 X 10 ¹ copies/μl			
Molecular Grade	1 Vials of 1 ml	1 Vial of 1 ml	1 Vial of 1 ml
Water.			
			A Vial of A well
IC-1 RG (R3)	1 Vial of 1 ml	1 Vial of 1 ml	1 Vial of 1 ml
	Dengue 1-4 Super mix. Mg Sol RT. Dengue 1-4 Standard 1 1 X 10 ⁵ copies/µl Dengue 1-4 Standard 2 1 X 10 ⁴ copies/µl Dengue 1-4 Standard 3 1 X 10 ³ copies/µl Dengue 1-4 Standard 4 1 X 10 ² copies/µl Dengue 1-4 Standard 5 1 X 10 ¹ copies/µl Molecular Grade Water.	100 rxnsDengue 1-4 Super mix.25 rxns x 4 VialsMg Sol RT.1 VialDengue 1-4 Standard 1 1 X 105 copies/µl1 Vial of 300µlDengue 1-4 Standard 2 1 X 104 copies/µl1 Vial of 300µlDengue 1-4 Standard 3 1 X 103 copies/µl1 Vial of 300µlDengue 1-4 Standard 3 1 X 103 copies/µl1 Vial of 300µlDengue 1-4 Standard 4 1 X 102 copies/µl1 Vial of 300µlDengue 1-4 Standard 4 1 X 102 copies/µl1 Vial of 300µlStandard 5 1 X 101 copies/µl1 Vial of 300µlMolecular Grade Water.1 Vials of 1 ml	100 rxns50 rxnsDengue 1-4 Super mix.25 rxns x 4 Vials25 rxns x 2 VialsMg Sol RT.1 Vial1 VialDengue 1-4 Standard 1 1 X 10 ⁵ copies/µl1 Vial of 300µl1 Vial of 300µlDengue 1-4 Standard 2 1 X 10 ⁴ copies/µl1 Vial of 300µl1 Vial of 300µlDengue 1-4 Standard 3 1 X 10 ³ copies/µl1 Vial of 300µl1 Vial of 300µlDengue 1-4 Standard 3 1 X 10 ³ copies/µl1 Vial of 300µl1 Vial of 300µlDengue 1-4 Standard 4 1 X 10 ² copies/µl1 Vial of 300µl1 Vial of 300µlDengue 1-4 Standard 5

1. Contents of the Kit:

R = Reagents

S = Quantitation Standards

W = Molecular Grade Water.

All Vials have Color Coder tops to distinguish between different reagents.

2. Storage of the Kit.

All the reagents of the kit should be stored at -20°C and is stable till expiry at this temperature. Repeated thawing and freezing (> 3x) should be avoided, as this may reduce the sensitivity of the assay. If the kit is to be used only occasionally, the reagents should be frozen in aliquots. Storage at +4°C is not recommended & should not exceed a period of 2 hours in any case.

3. Dengue 1-4 Information

Application:

Dengue is a flu-like viral disease spread by the bite of *Aedes* infected mosquitoes. Dengue hemorrhagic fever is a severe, often fatal, and lead to several complications. Dengue occurs in most tropical areas of the world.

There is no specific treatment for dengue. Prevention centers on avoiding mosquito bites in areas where dengue occurs or might occur and eliminating breeding sites. Dengue fever usually starts suddenly with a high fever, rash, severe headache, pain behind the eyes, and muscle and joint pain. The severity of the joint pain has given dengue the name "breakbone fever." Nausea, vomiting, and loss of appetite are common. A rash usually appears 3 to 4 days after the start of the fever. The illness can last up to 10 days, but complete recovery can take as long as a month. Older children and adults are usually sicker than young children. Most dengue infections result in relatively mild illness, but some can progress to dengue hemorrhagic fever. With dengue hemorrhagic fever, the blood vessels start to leak and cause bleeding from the nose, mouth, and gums. Bruising can be a sign of bleeding inside the body. Without prompt treatment, the blood vessels can collapse, causing shock (dengue shock syndrome). Dengue hemorrhagic fever is fatal in about 5 percent of cases, mostly among children and young adults. There are four different distinct sero types namely Dengue 1, Dengue 2, Dengue 3 & Dengue 4. All four dengue serotypes produce clinically identical disease, and all can produce DHF and dengue shock syndrome (DSS) in decreasing order of frequency: serotypes 2, 3, 4, and 1. Individuals infected with one strain maintain lifelong homotypic immunity while remaining susceptible to infections with other heterotypic strains. Interestingly, DHF/DSS is more likely to develop if an individual previously infected with one serotype is later inoculated with a different viral strain. DHF and DSS usually occur as a second dengue infection in children and in infants born to dengue-immune mothers. repeated episodes of DHS/DSS have not been described in the same individual.

The Dengue Serotypes

Dengue infections are caused by four closely related viruses named DEN-1, DEN-2, DEN-3, and DEN-4. These four viruses are called serotypes because each has different interactions with the antibodies in human blood serum. The four dengue viruses are similar — they share approximately 65% of their genomes — but even within a single serotype, there is some genetic variation. Despite these variations, infection with each of the dengue serotypes results in the same disease and range of clinical symptoms.

Are these four viruses all found in the same regions of the world? In the 1970s, both DEN-1 and DEN-2 were found in Central America and Africa, and all four serotypes

4

were present in Southeast Asia. By 2004, however, the geographical distribution of the four serotypes had spread widely. Now all four dengue serotypes circulate together in tropical and subtropical regions around the world (Figure 1). The four dengue serotypes share the same geographic and ecological niche. Where did the dengue viruses first come from? Scientists hypothesize that the dengue viruses evolved in nonhuman primates and jumped from these primates to humans in Africa or Southeast Asia between 500 and 1,000 years ago

After recovering from an infection with one dengue serotype, a person has immunity against that particular serotype. Does infection with one serotype protect against future dengue infections with the other serotypes? Individuals are protected from infections with the remaining three serotypes for two to three months after the first dengue infection. Unfortunately, it is not long-term protection. After that short period, a person can be infected with any of the remaining three dengue serotypes. Researchers have noticed that subsequent infections can put individuals at a greater risk for severe dengue illnesses than those who have not been previously infected.

Dengue Virus Genome and Structure;

The dengue virus genome is a single strand of RNA. It is referred to as *positive-sense RNA* because it can be directly translated into proteins. The viral genome encodes ten genes (Figure 2). The genome is translated as a single, long polypeptide and then cut into ten proteins.

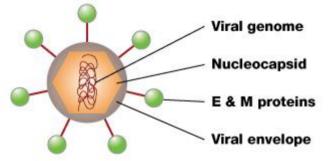


FIG.1

The dengue virus has a roughly spherical shape. Inside the virus is the nucleocapsid, which is made of the viral genome and C proteins. The nucleocapsid is surrounded by a membrane called the viral envelope, a lipid bilayer that is taken from the host. Embedded in the viral envelope are E and M proteins that span through the lipid bilayer. These proteins form a protective outer layer that controls the entry of the virus into human cells.

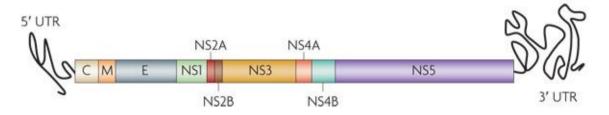


FIG.2

What are the roles of these ten proteins? Three are structural proteins: the capsid (C), envelope (E), and membrane (M) proteins. Seven are nonstructural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. These nonstructural proteins play roles in viral replication and assembly.

The structure of the dengue virus is roughly spherical, with a diameter of approximately 50 nm (1 nm is one millionth of 1 mm) (Figure 1). The core of the virus is the nucleocapsid, a structure that is made of the viral genome along with C proteins. The nucleocapsid is surrounded by a membrane called the viral envelope, a lipid bilayer that is taken from the host. Embedded in the viral envelope are 180 copies of the E and M proteins that span through the lipid bilayer. These proteins form a protective outer layer that controls the entry of the virus into human cells.

Once the virus has entered a host cell, the virus penetrates deeper into the cell while still inside the endosome. How does the virus exit the endosome, and why? Researchers have learned that two conditions are needed for the dengue virus to exit the endosome:

1. The endosome must be deep inside the cell where the environment is acidic.

2. The endosomal membrane must gain a negative charge.

These two conditions allow the virus envelope to fuse with the endosomal membrane, and that process releases the dengue nucleocapsid into the cytoplasm of the cell.

Once it is released into the cell cytoplasm, how does the virus replicate itself? In the cytoplasm, the nucleocapsid opens to uncoat the viral genome. This process releases the viral RNA into the cytoplasm. The viral RNA then hijacks the host cell's machinery to replicate itself. The virus uses ribosomes on the host's rough endoplasmic reticulum (ER) to translate the viral RNA and produce the viral polypeptide. This polypeptide is then cut to form the ten dengue proteins.

The newly synthesized viral RNA is enclosed in the C proteins, forming a nucleocapid. The nucleocapsid enters the rough ER and is enveloped in the ER membrane and surrounded by the M and E proteins. This step adds the viral envelope and protective outer layer. The immature viruses travel through the Golgi apparatus complex, where the viruses mature and convert into their infectious form. The mature dengue viruses are then released from the cell and can go on to infect other cells.

The *Geno-Sen's* Dengue 1-4 Quantification assay is developed for laboratory scale or high-throughput quantitative transcript analysis by real time quantitative fluorescence PCR. Geno Sen's standardized ready-to-use Control and Reaction mix allow fast processing of the samples.

Samples which can be used for Extraction: Serum, plasma, whole blood & C.S.F., Cell culture, etc.

4. Precautions for PCR

The following aspects should always be taken care of:

- Store positive material (Specimens, Standards or amplicons) separately from all other reagents and add it to the reaction mix in a separate facility.
- Thaw all components thoroughly at room temperature before starting the assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice or in the Cooling Block.
- All the reagents including the NTC (except for standards & specimens) should be mixed & dispensed in pre-mix area.
- All the standards & specimens should be mixed & dispensed in extraction area.
- Use pipette tips with filters only.
- Always use disposable powder-free gloves

5. Additionally Required Materials and Devices

- RNA isolation kit (see 8.a. RNA extraction)
- 0.2 ml PCR tubes for use with 36-well rotor (Corbett Research, Cat.-Nr.: SE-1003F) alternatively 0.1 ml PCR tubes for use with 72-well rotor (Corbett Research, Cat.-Nr.: ST-1001)
- Micro Pipettes Variable Volume 2-20µl, 10-100µl, 100-1000µl,
- Sterile pipette tips with aerosol barrier 2-20µl, 10-100µl, 100-1000µl,
- Disposable powder-free gloves
- Vortex mixer
- Centrifuge Desktop with rotor for 1.7 ml reaction tubes
- Rotor Gene[™] 2000,3000 or Rotor Gene[™] 6000, Corbett Research (The Real time PCR Instrument)

6. Principle of Real-Time PCR

The robust assay exploits the so-called Taqman principle. During PCR, forward and reverse primers hybridize to a specific sequence product. A TaqMan probe, which is contained in the same reaction mixture and which consists of an oligonucleotide labeled with a 5'-reporter dye and a downstream, 3'-quencher dye, hybridizes to a target sequence within the PCR product. A Taq polymerase which possesses 5' - 3' exonuclease activity cleaves the probe. The reporter dye and quencher dye are separated upon cleavage, resulting in an increase in fluorescence for the reporter. Thus, the increase in fluorescence is directly proportional to the target amplification during PCR.

7. Description Of the Product.

The **Geno-Sen's** Dengue **1-4** *PCR* Reagents constitute a ready to use system for detection and quantification of Dengue 1-4 using Polymerase chain reaction (PCR) in the Rotor Gene 2000/3000/6000 (Corbett Research). The Specific Master mix contains reagents and enzymes for the specific amplification of Dengue 1-4 and for the direct detection of the specific amplicon in fluorescence channel Cycling A.FAM of the Rotor Gene 2000/3000/6000 & the Reference gene on Cycling A. Joe. External positive Standards (Dengue 1-4 S 1-5) are supplied which allow the determination of the gene load. For further information, please refer to section 8.c Quantitation.

8. Procedure

8.a RNA Extraction

RNA Extraction kits are available from various manufacturers. Sample volumes for the RNA Extraction procedure depend on the protocol used. Please carry out the RNA Extraction according to the manufacturer's instructions. The recommended Extraction kits are the following:

Sample	Nucleic Acid	Cat. Num.	
Material	Isolation Kit	REF	
Serum or plasma.C. S.F.etc.	Geno Sen's [®] Viral RNA Extraction Mini Kit (Columns based)	98001 or 98002	Genome Diagnostics Pvt. Ltd. India.

The **Geno Sen's**[®] Dengue Real Time PCR kits has been optimized with the above mentioned extraction kit. The **Geno Sen's**[®] Viral RNA Extraction Mini Kit provides a relatively higher yield than most of the commercial extraction kits available on the world market & hence is the preferred Kit for extraction of Viral RNA. However the customer can use their own extraction systems depending on how good the yield is. Always use an extraction kit with a higher RNA yield otherwise the low positives will not be detected.

However the customer can use their own extraction systems depending on how good the yield is. Always use an extraction kit with a higher RNA yield.

Blood collection tubes coated with anticoagulants may inhibit the PCR, However these inhibitors will be eliminated by the use of the isolation kits given above. It is recommended to avoid the usage of heparin blood.

When using Extraction protocols with ethanol-containing washing buffers, please carry out an additional centrifugation step before the elution to remove any remaining ethanol. This prevents possible inhibition of PCR.

The Dengue *1-4 Rotor Gene PCR Reagents* should not be used with phenol based isolation methods.

Samples which can be used for extraction are: Whole Blood, Serum, C.S.F etc.

8.b *Inhibition Control:*

Inhibition Control Gene allows the user to determine & control possible PCR inhibition. The Inhibition Control gene reagents are in built in the premix provided and need not be run separately. There is need to add internal control Gene in the reaction mix which has been provided as IC-1 RG (R3). To add the IC Gene 0.5 μ I/rxn of R3 is added to the premix as shown in the Figure 4 & 5. The results can be visualized in the Joe channel.

8.c Quantitation

The quantitation standards provided in the kit (Dengue 1-4 S 1-5) are treated in the same way as extracted samples and the same volume is used i.e. (15μ) instead of the sample. To generate a standard curve in the *RotorGene*TM 2000/3000/6000, all 5 Standards should be used as defined in the menu window *Edit Samples* of the *RotorGene*TM software. The same should also be defined as standards with the specified concentrations (see *RotorGene*TM Manual). The standard curve generated as above can also be used for quantitation in subsequent runs, provided that at least one standard is used in the current run. For this purpose, the previously generated standard curve needs to be imported (see *Rotor Gene*TM 2000/3000/6000 Manual). However, this quantitation method may lead to deviations in the results due to variability between different PCR runs & due to varying Reaction efficiencies.

<u>Attention</u>: The standards are defined as Copies/µl. The following formula has to be applied to convert the values determined using the standard curve into Copies/ml of sample material:

Result (Copies/µl) x Elution Volume (µl)

Result (Copies/ml) =

Sample Volume (ml)

Or else direct conversion can be done keeping in mind the starting volume of the sample & the final eluted Volume e.g.

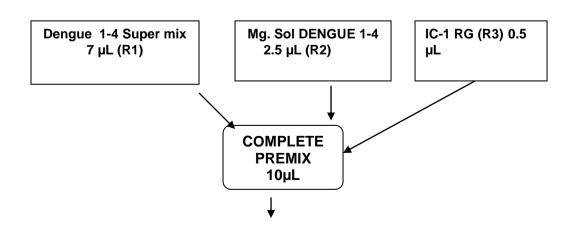
In case *Geno Sen's*[®] Viral RNA Extraction Mini Kit is being used where the starting volume is 150µl & the final Eluted Volume is 60µl then to obtain the direct values i.e. copies/ml for the patient samples the following values for the standards can be fed directly into the operating software which is again based on the above formula and will yield direct conversion of per ml of patient sample data.

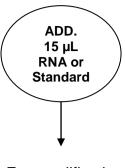
S1: 10 ⁵ copies /μl =	40000000 copies /ml
S2: 10 ⁴ copies /μl =	4000000 copies /ml
S3: 10 ³ copies /μl =	400000 copies /ml
S4: 10 ² copies /μl =	40000 copies /ml
S5: 10 ¹ copies /μl =	4000 copies /ml

8.d Preparation for PCR & amplification.

First make sure that the Cooling Block (accessory of the *Rotor Gene*TM, Corbett Research) is pre-cooled to +4°C in a Refrigerator or Deep Freezer. Place the desired number of PCR tubes into the Cooling Block. Make sure that the tubes for standards & at least one negative control (*Water, PCR grade*) are included per PCR run. To generate a standard curve, use all supplied Standards (Dengue 1-4, S 1-5) for each PCR run. Before each use, all reagents need to be thawed completely and mixed (by pipetting or by brief vortexing).

Please follow the pipeting scheme mentioned below for each sample depending upon the number of samples a mix can be prepared as follows. Each sample.





For amplification

Fig. 4.

Depending upon the number of samples the following pipetting scheme can be followed. e.g. for 10 rxns.

Dengue 1-4	1 rxns.	10 rxns.
MASTER MIX		
Dengue 1-4 Super	7 μL	70 µL
Mix (R1)		
Dengue 1-4 Mg	2.5 μL	25 µL
Sol. (R2)		
IC-1 RG (R3)	0.5 μL	5 µL
Total	10µL	100µL

Fig. 5.

Pipette 10 µl of the Master Mix into each labelled PCR tube. Then add 15 µl of the earlier extracted RNA to each sample tube and mix well by pipeting up and down. Correspondingly, 15 µl of the Standards (DENGUE 1-4 *S1-5*) must be used as a positive control and 15 µl of water (*Water, PCR grade*) as a negative control. Close the PCR tubes and transfer the same into the rotor of the *RotorGene*TM instrument. The *RotorGene*TM software versions 5.0.53 and higher require a Locking Ring (accessory of the *RotorGene*TM, Corbett Research) to be placed on top of the rotor to prevent accidental opening of the tubes during the run.

8.e. Programming the *RotorGene*[™] 2000/3000

The *RotorGene*[™] 2000/3000 PCR program for the detection of DENGUE 1-4 can be divided into following steps:

- A. Setting of general assay parameters & reaction volume
- B. Thermal Profile & Calibration
- C. Cycling profile/ cDNA synthesis & Initial activation of the Hot Start enzyme
- D. Cycling for Amplification of cDNA
- E. Adjustment of the sensitivity of the fluorescence channels

F. Starting of the Rotor Gene™ run

Program the *RotorGene*TM 2000/3000 for these 5 steps according to the parameters shown in Fig. 6-21. All specifications refer to the *RotorGene*TM software version 6.0.33. Please find further information on programming the *RotorGene*TM in the *RotorGene*TM 2000/3000 Operator's Manual,. In the illustrations these settings are shown by arrows

Setting of general assay parameters & Reaction volume.

First, confirm whether the PCR tubes used are No Domed PCR tubes by clicking in the box as shown in the figure. Please note that tubes with domes cannot be used in Rotor gene hence click in the box as shown below.

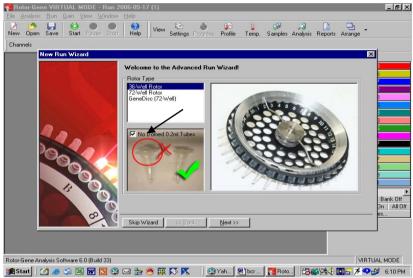


Fig. 6.

Confirmation of Reaction Volume as follows.

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New Run Wizard	
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	23 24 ■ Bank On Bank Off Named On All On All Off Edit Samples
Rotor-Gene Analysis Software 6.0 (Build 33)	VIRTUAL MODE

Fig. 7. Setting of general assay parameters.

- Please click on the volume buttons to make sure that 25µl is reflected in the window as shown above.
- Then click next and a new window will open as shown below.

THERMAL PROFILE & CALIBRATION:

Here the thermal profile for the assay will be defined.

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Fig. 8.

Programming the temperature profile is done by activating the button *Edit Temperature Profile* in the next *New Experiment Wizard* menu window as shown above.

CYCLING PROFILE: First hold 50°C for 15 minutes i.e. cDNA synthesis step as below

💦 Rotor-	Gene VIRTUAL MODE - Run 2006-05-20 (1)	_ 8 ×
	😰 Edit Profile	
New 0	New Open Save As Help	
Channel	The run will take approximately 115 minute(s) to complete. The graph below represents the run to be performed :	
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	Hold Temperature : 50 K	
	Hold Time : 15 mins 0 secs	
	Rotor Speed : Normal Speed .	
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Fig. 9.

CYCLING PROFILE: Second hold 95°C for 10 minutes as below

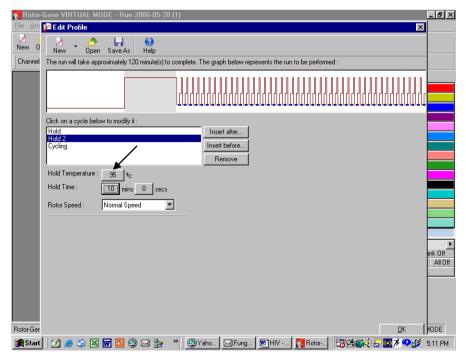


Fig. 10. Initial activation of the Hot Start enzyme.

Setting up of denaturation step in the cycling profile as depicted below i.e.

95°C for 15 seconds.

	Gene VIRTUAL MODE - Run 2006-09-09 (1)	_ 8 ×
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New 0	New Open Save As Help	
Channel	The run will take approximately 115 minute(s) to complete. The graph below represents the run to be performed :	
		3L
	Click on a cycle below to modify it :	
	Hold Insert after Evalue Bysing Bernove Remove	
	This cycle repeats 45 time(s).	
	Click on one of the steps below to modify it, or press + or - to add and remove steps for this cycle.	
	Timed Step ▼ 95 ⁸ c 15 seconds Not Acquiring Normal Speed ▼ Cong Range Touchdown	nk Off All Off -
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	Fig. 11.	

Setting up of Anneling step in the cycling profile as depicted below i.e. 55°C for



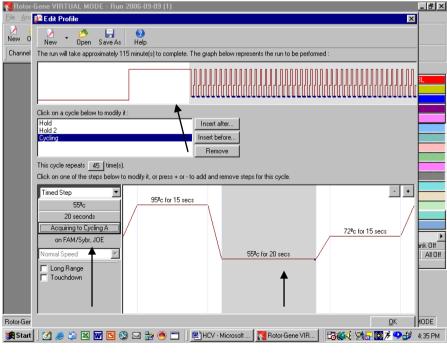


Fig. 12.

Setting up of Extension step in the cycling profile as depicted below i.e. 72°C for 15 Seconds

💦 Rotor-O	Gene VIRTUAL MODE - Run 2006-09-09 (1)
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Channel	The run will take approximately 115 minute(s) to complete. The graph below represents the run to be performed :
	Click on a cycle below to modify it : Hold Hold 2 Ursett after Gveling Remove
	This cycle repeats 45 time(s). Click on one of the steps below to modify it, or press + or - to add and remove steps for this cycle.
	Timed Step 95% for 15 secs 72% c 15 seconds Not Acquiring 72% for 15 secs Normal Speed 72% for 15 secs Long Range 55% for 20 secs
Rotor-Ger	OK HODE
Fig.	

Setting up of Number of Cycles to 45 cycles in the cycling profile as depicted

below.

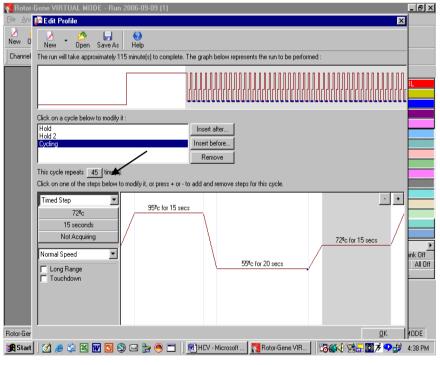


Fig. 14.

Final Confirmation of the Thermal profile by pressing OK button as shown below.

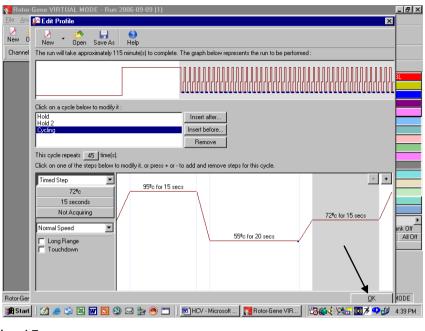


Fig. 15.

Setting the gains for the acquiring channel by clicking at the Calibrate button as shown below.

Rotor-Gene VIRTUAL MODE - Run 2006-05-20 (1) File Analysis Run Gain View Window Help		
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Rotor-Gene Analysis Software 6.0 (Build 33)	@Vala @I.m. @U.M.	
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Fig. 16.

The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. This adjustment is done in the menu window *Auto Gain Calibration*

Setup (activation in menu window New Experiment Wizard under Calibrate). Please set the calibration temperature to the annealing temperature of the amplification program (compare Fig. 16).

Adjustment of the fluorescence channel sensitivity as shown below.

▼ Rotor-Gene VIRTUAL MODE - Run 2006-10-25 (1) File Analysis Run Gain View Window Help	
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Start: Manual Close Help	Bank Off Dn All Off es
Rotor-Gene Analysis Software 6.0 (Build 33)	VIRTUAL MODE
	\$€\$€\$ © : : : : : : : : : :
Fig. 17.	
Rotor-Gene VIRTUAL MODE - Run 2006-10-25 (1)	_ B ×
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Channels Auto Gain Calibration Setup	
New Run Wizard Calibration : Auto-Gain Calibration will read the fluorescence on the inserted sample at different gain levels until it finds one at which the fluorescence levels are acceptable. The range of fluorescence you are looking for depends on the chemistry you are performing. Set temperature to 50 million and the fluorescence in the inserted sample at librate All Calibrate Acquiring Perform Calibration Before 1st Acquisitori Perform Calibration At 55 Degrees At Beginning Of Run Channel Settings : Add Name Tube Position Manee Tube Position	This box displays help on elements in the wizard. For help on an item, hover your mouse over the item for help. You can also citics on a combo box to display available settings.
FAM/Sybr 1 5FI 10FI JOE 1 5FI 10FI Remove All Start Manual	Bank Off Dn All Off es
FAM/Sybr 1 5FI 10FI JOE 1 5FI 10FI Remove All Remove All	Dn All Off es VIRTUAL MODE

Fig. 18.

Please do not forget to click on the box against "Perform calibration Before 1st acquisition." After that press Close and a new window will open as shown below.

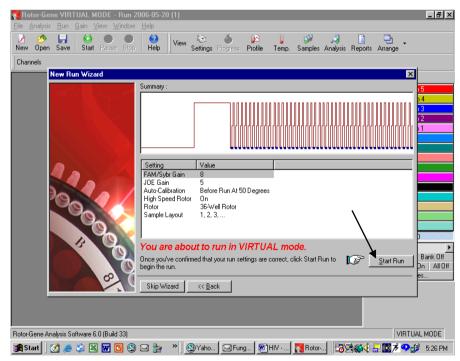
The gain values determined by the channel calibration are saved automatically and are listed in the last menu window of the programming procedure.

PRESS NEXT

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Fig. 19.

Starting of the Rotor Gene™ run.





Press Start Run Button.

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Rotor-Gene Analysis Software 6.0 (Build 33) VIRTU. ■Start 2 @ @ X W © © © > ● ○ 1 0 @MT @DE @Den Rot C&&&& = 0 @ # Fig. 21.	AL MODE

SAVING THE RUN FILE AS ABOVE

Store the run file either in my documents or a designated folder as shown above.

The moment save button is clicked after the file name, machine will start.

8.f. Programming the *Rotor Gene*[™] 6000

The *RotorGene*[™] 6000 PCR program for the detection of Dengue 1-4 can be divided into following steps:

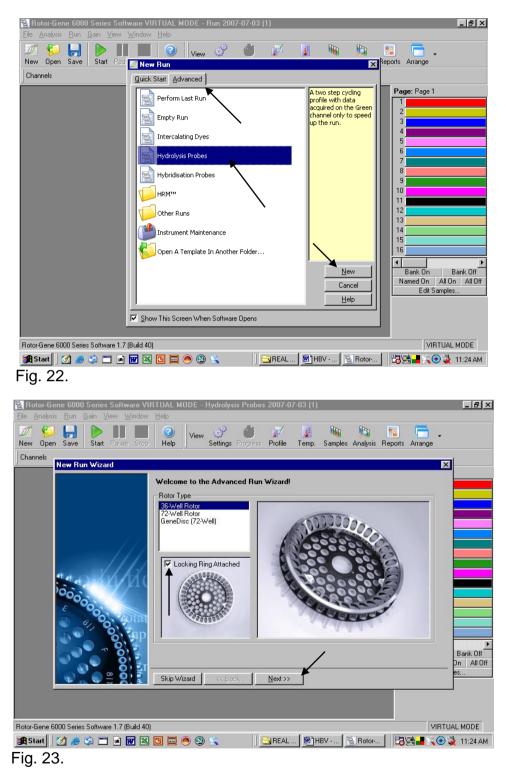
- G. Setting of general assay parameters & reaction volume
- H. Thermal Profile & Calibration
- I. Cycling profile/ cDNA Synthesis & Initial activation of the Hot Start enzyme
- J. Cycling for Amplification of cDNA
- K. Adjustment of the sensitivity of the fluorescence channels
- L. Starting of the Rotor Gene™ run

Program the *RotorGene*TM 6000 for these 5 steps according to the parameters shown in Figures 22-39 below All specifications refer to the *RotorGene*TM 6000 software version 1.7 Please find further information on programming the *RotorGene*TM in the *RotorGene*TM 6000 *Operator's Manual*, In the illustrations these settings are shown by arrows

g) Setting of general assay parameters & Reaction volume.

Please see to it that you are in advanced mode and then click Hydrolysis Probes.

On a double click the software will automatically go to the next function. If there is just a single click then click new after highlighting the Hydrolysis Probes.



First, confirm by clicking in the box as shown in the figure above whether a locking ring has been attached. Then click next to proceed to the next step.

😫 Rotor-Gene 6000 Series Software VIRTUAL MODE - Run 2008-06-09 (1)	🗖 🗗 🗙
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	Bank On Bank Off Named On All On All Off Edit Samples
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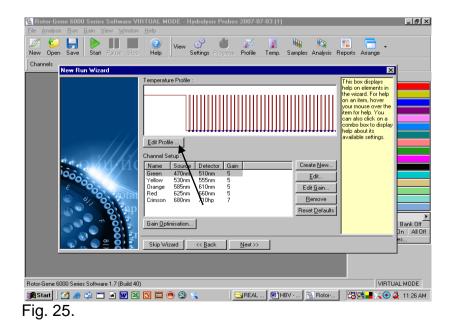
Confirmation of reaction Volume as follows.

Fig. 24.

- Please click on the volume buttons to make sure that 25µl is reflected in the window as shown above.
- In case required, Operators Name can be Fed into the system.
- Any Notes which need to be either printed into the Report can be typed out in the Box of Notes.
- Then click next and a new window will open as shown below.

h) THERMAL PROFILE & CALIBRATION:

Here the thermal profile for the assay will be defined.



Programming the temperature profile is done by activating the button *Edit Profile* in the next *New RUN Wizard* menu window as shown above.

i) CYCLING PROFILE: First hold 50°C for 15 minutes i.e. cDNA synthesis step as below

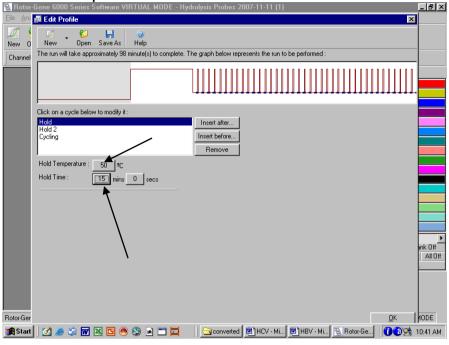


Fig. 26.

Second hold 95°C for 10 minutes as below

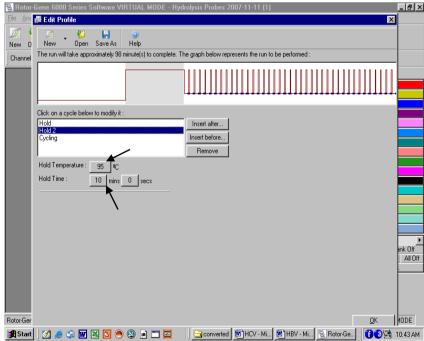


Fig. 27. Initial activation of the Hot Start enzyme. Generally the window will open with the Hold Temp as 95°C and the Hold Time as 10 minutes. In case it is different then set the same to as shown above.

j) Setting the Cycling and acquisition.

When clicked on Cycling the window will open as below.

😫 Rotor-	Gene 6000 Series Software VIRTUAL MODE - Hydrolysis Probes 2007-11-11 (1)	
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Mew 0	New Open Save As Help	
Channel	The run will take approximately 98 minute(s) to complete. The graph below represents the run to be performed :	
	, Click on a cycle below to modify it :	
	Hold Insert after Eveling Insert before	
	Remove	
	This cycle repeats 40 time(s). Click on one of the steps below to modify it, or press + or - to add and remove steps for this cycle.	
	Timed Step 95%C for 10 secs 95%C 95%C for 10 secs 10 seconds Not Acquiring F Long Range Touchdown 60%C for 45 secs	
Rotor-Ger		<u>O</u> K IODE
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Fig. 28.

Click on the Plus sign at the right hand as shown in the figure to make the Cycling a three step process. A new window as shown below will be there.

Setting up of denaturation step in the cycling profile as depicted below i.e. 95°C for 15 seconds.

S Rotor-Gene 6000 Series Software VIRTUAL MUDE - Hydrolysis Probes 2007-11-11 [1]	_ <u>– b ×</u>
Elle Ana M Edit Profile	×
New D New Open Save As Help	
Channel The run will take approximately 115 minute(s) to complete. The graph below represents the run to be performed :	
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This cycle repeats 40 time(s).	
Click on one of the steps below to modify it, or press + or - to add and remove steps for this cycle.	
Timed Step 95%C for 15 secs 95%C 15 seconds Not Acquiring 72%C for 20 secs Cong Range 60%C for 45 secs	nk Off
Rotor-Ger <u>D</u> K	HODE
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Fig. 29.

Setting up of Anneling step in the cycling profile as depicted below i.e. 55°C for 20 Seconds

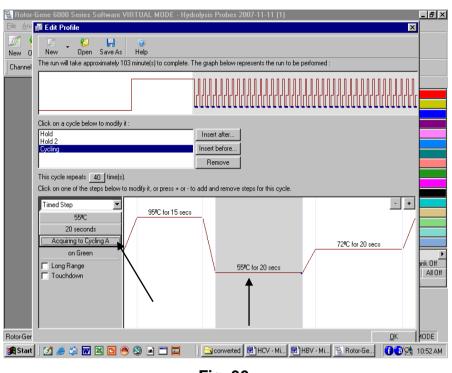


Fig. 30.

After setting the Anneling temperature and the time for anneling click on the "Acquiring to Cycling A" as shown by arrow. A New window will open as shown below.

Defining the Data acquiring channel i.e Green (FAM) & Yellow (JOE)

😫 Rotor-l	Gene 6000 Seri	es Softwa	re VIRTI	JAL MODE	: - Hydrolysis Probes 2007-11-11 (1)		_ 🖻 🗡
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					ect it from the list in the left and click >. To stop acquiring from a		
	This cycle repeat	channel,	select it ir	n the right-ha	nd list and click <. To remove all acquisitions, click <<.		
	Click on one of th						
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	55º					1	
	20 sec			Detector		/	
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	on Gr	Yellow	530nm	555nm	JOE [®] , VIC [®] , HEX, TET [®] , CAL Fluor Gold 540 [®] , Yakima Yellow [®]		▶ ank Off
	C Long Range	Orange	585nm	610nm	ROX ¹ , CAL Fluor Red 610 ¹ , Cv3.5 ¹ , Texas Red ¹ , Alexa Fluor 568 ¹		All Off
	1 rodendown	Red	625nm	660nm	Cy5 ¹ , Quasar 670 ¹ , LightCycler Red640 ¹ , Alexa Fluor 633 ¹		
		Crimson	680nm	710hp	Quasar705 ¹ , LightCycler Red705 ¹ , Alexa Fluor 680 ¹		
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					Fig. 31.		

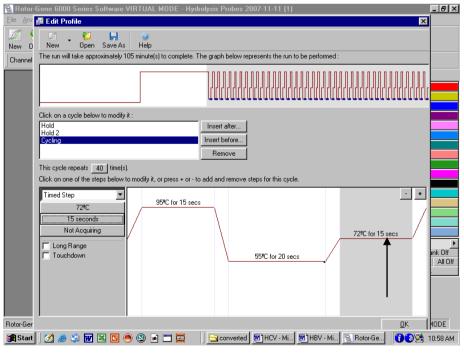
Highlight the Yellow and then press the right arrow. Just see before shifting the yellow to right that there is no other channel in the right except for Green. In case any other Channel appears besides Green on the right then the same be shifted to the left.

🙀 Rotor-Gene 6000 Series Software VIRTUAL MODE - Hydrolysis Probes 2007-11-11 (1)	- 8 ×
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Channel The run will take Acquisition	
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Acquisition Configuration :	
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This cycle repeat channel, select it in the right-hand list and click <. To remove all acquisitions, click <<.	
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	ank Off
Touchdown Orange 585nm 610nm ROX [®] , CAL Fluor Red 610 [®] , Cy3.5 [®] , Texas Red [®] , Alexa Fluor 568 [®]	All Off
625nm 660nm Cy5 ³ , Quasar 670 ³ , LightCycler Red640 ³ , Alexa Fluor 633 ³	
Crimson 680nm 710hp Quasar705 [®] , LightCycler Red705 [®] , Alexa Fluor 680 [®]	
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Confirmation of Channels as shown below.

Fig. 32.

Once the Yellow and Green Channels are on the Right side then press OK as shown by the arrow. Setting up of Extension step in the cycling profile as depicted below i.e. 72°C for 15



Seconds

Fig. 33.

Setting up of Number of Cycles to 45 cycles in the cycling profile as depicted below.

😫 Rotor-l	Gene 6000 Series Software VIRTUAL MODE - Run 2007-11-11 (1)	_ 8 ×
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<u>,</u>		
	Click on a cycle below to modify it :	
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	This cycle repeats 45 times	
	Click on one of the steps below to modify it, or press + or - to add and remove steps for this cycle.	
DueCo	Timed Step 95% C for 15 secs 15 seconds 72% C for 15 secs Not Acquiring 72% C for 15 secs Touchdown 55% C for 20 secs	ank Off
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	Fig. 34.	

After setting the number of Cycles Press OK.

k) Setting the gains for the acquiring channel by clicking at the Gain Optimisation

button as shown below.

		RTUAL MODE - Run 2007-11-11 (1)	_ & ×
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The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. This adjustment is done in the menu window *Auto Gain optimization*

Setup (activation in menu window New Experiment Wizard under Gain Optimization). Please set the temperature to the annealing temperature of the amplification program (compare Fig.36.

🔁 Rotor-Gene 6000 Series Software VIRTUAL MODE - Run 2007-11-11 (1)	_ 8 ×
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Fig. 36. The following needs to be done.

- Click on the Optimise Acquiring
- Click on Set temperature To Adjust the Temperature to 55°C.
- Click on the Box Perform Optimisation before 1st Acquisition.
- Just see that below the channel settings there appear only two channels i.e. Green and Yellow. In case it is different then the channels have not been set properly. Close the window and reset the channels.
- Then Press Close.
- The press Next as shown below.

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New Hun Wizard		
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Fig. 37.

L) PRESS Start RUN

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Fig. 38.

Saving the RUN File.

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Save as type: Run File (".rex)	
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Once you've confirmed that your run settings are correct, click Start Run to begin the run. Click Save Template to save settings for future runs.	Date Bank Off Dn All Off
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Fig. 39.	

Store the run file either in my documents or a designated folder as shown above. The moment save button is clicked after the file name, machine will start.

9. a) Generated Data Interpretation & Analysis for 2000/3000

Data analysis is performed with the *RotorGene*[™] software according to the manufacturer's instructions (*RotorGene*[™] 3000 Operator's Manual).

The following results are possible:

A signal is detected in fluorescence channel Cycling A.FAM.

The result of the analysis is positive: The sample contains Dengue 1-4 RNA. In fluorescence channel Cycling A.FAM no signal is detected.

No Dengue 1-4 RNA detectable. It can be considered negative if there is amplification in the Joe Channel. Please refer for details on PCR inhibition in the section PCR inhibition.

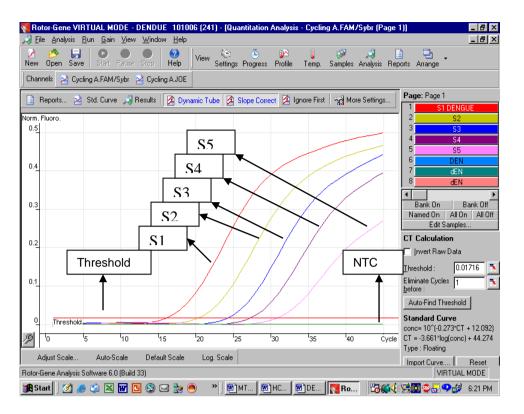


Fig. 40: Detection of the quantitation standards (*Dengue 1-4 S 1-5*) in fluorescence channel Cycling A.FAM. NTC: non-template control.

Examples of positive and negative PCR reactions are given in the above figure.

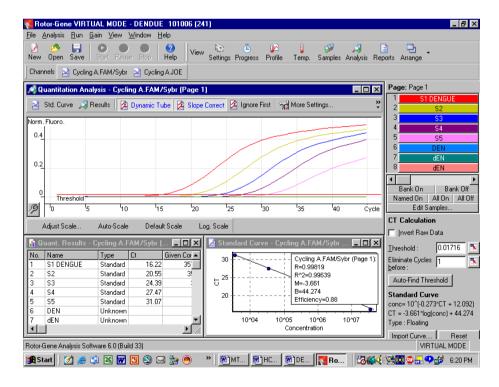


Fig. 41.

Example of analysed data for Dengue 1-4 where all the amplification curves can be seen as well as the standard curve, reaction efficiency, M value etc. can be seen and the quantitative data for the samples can be seen.

Inhibition Control gene amplification.

Analyze the Joe Channel

PCR Inhibition

A signal is detected in fluorescence channel Cycling A. Joe: No PCR inhibition

In fluorescence channel Cycling A. Joe no signal is detected.

But signal detected in FAM: The sample is positive for Dengue 1-4 RNA.

In fluorescence channel Cycling A. Joe no signal is detected.

No signal detected in FAM as well: A possible PCR inhibition has occurred.

9. b) Generated Data Interpretation & Analysis for 6000

Data analysis is performed with the *RotorGene*[™] software according to the manufacturer's instructions (*RotorGene*[™] 6000 Operator's Manual).

The following results are possible:

A signal is detected in fluorescence channel Cycling A.Green.

The result of the analysis is positive: The sample contains Dengue 1-4 RNA.

In fluorescence channel Cycling A.Green no signal is detected.

No Dengue 1-4 RNA detectable. It can be considered negative if there is amplification in the Yellow Channel. Please refer for details on PCR inhibition in the section PCR inhibition.

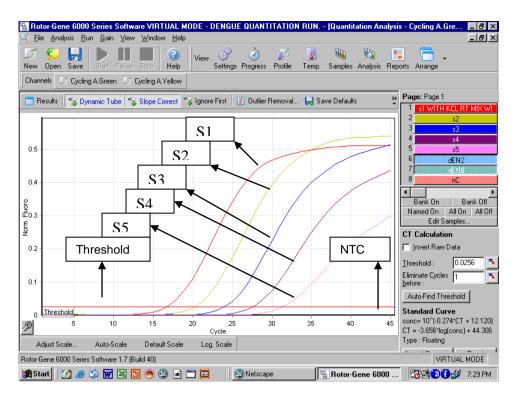


Fig. 42: Detection of the quantitation standards (*Dengue 1-4 S 1-5*) in fluorescence channel Cycling A.Green. NTC: non-template control.

Examples of positive and negative PCR reactions are given in the above figure.

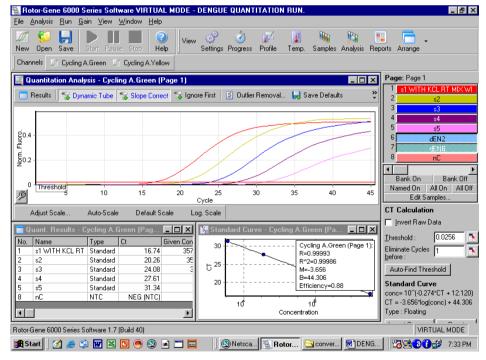


Fig. 43.

Example of analysed data for Dengue 1-4 where all the amplification curves can be seen as well as The standard curve, reaction efficiency, M value etc. can be seen and the quantitative data for the samples can be seen.

Inhibition Control gene amplification.

Analyze the Yellow Channel

PCR Inhibition

A signal is detected in fluorescence channel Cycling A. Yellow: No PCR inhibition

In fluorescence channel Cycling A. Yellow no signal is detected.

But signal detected in Green: The sample is positive for Dengue 1-4 RNA.

In fluorescence channel Cycling A. Yellow no signal is detected.

No signal detected in Green as well: A possible PCR inhibition has occurred.

10. Troubleshooting

a) For Rotor gene 2000/3000

1. No signal with positive Standards (Dengue 1-4 S 1-5) in fluorescence channel Cycling A.FAM.

- Incorrect programming of the *Rotor-Gene*[™] 2000/3000.
 - → Repeat the PCR with corrected settings.

2. Weak or no signal in fluorescence channel Cycling A. FAM:

• The PCR conditions do not comply with the protocol.

- The DENGUE 1-4 Super Mix *R1* has been thawed and frozen too often.
- The DENGUE 1-4 Super Mix R1 has been kept at +4°C for longer than 5 hours.
 - → Please mind the storage conditions given in the **Storage**.

→ Repeat the assay using a new Dengue 1-4 super mix (R1).

• The PCR was inhibited.

[→] Repeat the PCR with corrected settings.

Make sure that you use a recommended extraction method (see 8.a. RNA extraction) and stick closely to the manufacturer's instructions.

10 Troubleshooting

b) For Rotor gene 6000

1. No signal with positive Standards (Dengue 1-4 S 1-5) in fluorescence channel Cycling A.Green.

- Incorrect programming of the *Rotor-Gene*[™] 6000.
 - ➔ Repeat the PCR with corrected settings.

2. Weak or no signal in fluorescence channel Cycling A. Green:

- The PCR conditions do not comply with the protocol.
 - → Repeat the PCR with corrected settings.
- The DENGUE 1-4 Super Mix *R1* has been thawed and frozen too often.
- The DENGUE 1-4 Super Mix R1 has been kept at +4°C for longer than 5 hours.
 - → Please mind the storage conditions given in the **Storage**.
 - → Repeat the assay using a new Dengue 1-4 super mix (R1).

• The PCR was inhibited.

Make sure that you use a recommended extraction method (see 8.a. RNA extraction) and stick closely to the manufacturer's instructions.

11. Specifications

11.a Sensitivity and Reproducibility

In order to determine the sensitivity of the *Geno-Sen's* Dengue 1-4 Real Time PCR *Kit*, a dilution series has been set up from 10^7 down to 10^0 Copies/µl of Dengue 1-4

RNA and analyzed with the **Geno-Sen's** Dengue 1-4 Real Time PCR Kit. The assays were carried out on three different days in the form of 8-fold determinations. The results were determined by a probit analysis. The detection limit as determined for **Geno-Sen's** Dengue 1-4 Real Time PCR Kit is consistently 70 Copies/ml. This means that there is 95% probability that 70 copies/ml will be detected.

Analytical Sensitivity

Analytical Sensitivity in Conjunction with the *Geno Sen's*[®] Viral RNA Extraction Mini Kit for RNA purification (Cat . No. 98001) of the *Geno Sen's*[®] Dengue Real Time PCR RG Kit on ROTOR GRNR 3000/6000 was determined by Spiking a known negative Serum to a nominal 85 copies/ml. This was subjected to extraction using the *Geno Sen's*[®] Viral RNA Extraction Mini Kit — for RNA purification (Cat . No. 98001) eight times with starting volume of 150µl & elution volume of 60µl.

All the Eight extractions were then analyzed with the Geno Sen's[®] Dengue Real Time PCR RG Kit along with the standards & the NTC.

All the eight extractions were amplified correctly & the mean value obtained for the eight replicates was 84 copies/ml.

Hence Analytical Sensitivity in Conjunction with the *Geno Sen's*[®] Viral RNA Extraction Mini Kit — for RNA purification (Cat. No. 98001) of the *Geno Sen's*[®] Dengue *Real Time PCR RG* was determined to be 85 copies/ml.

11.b Specificity

The specificity of the **Geno Sen's**[®] **Dengue Real Time PCR RG Kit** is ensured by selection of very specific Primers & probes. This is also ensured by stringent reaction conditions. The primers & probes were blasted for possible homologies to all sequences published in the GeneBank. It was found that the primers & probes are very specific to all the genotypes of Dengue.

To further Validate the stringent data In order to check the specificity of the *Geno Sen's*[®] Dengue *Real Time PCR RG Kit*, different RNA & DNA listed below were analyzed with *Geno Sen's*[®] Dengue *Real Time PCR RG Kit*. None of these led to a positive signal with the *Geno Sen's*[®] Dengue *Real Time PCR RG Kit*.

Vericella Zoster Virus	Hepatitis B Virus	N. Meningitis
Human Herpes Virus 6	Hepatitis A Virus	S. Pneumonia
Epstein barr Virus	Hepatitis E Virus	JEV
Cytomagalovirus	HIV-1	Mycobacterium
		tuberculosis
Chlamydia pneumonia	HIV 2	Chikungunya
Parvovirus B 19	Hantaan Virus	Staphylococcus aureus
HCV	H. Influenza	Salmonella enteritidis
Leprosy	Malaria	Scrub typhus
B.pseudomallie	West Nile Virus	Leptospira interrogans.
TTV	EnteroVirus	

In order to validate the Specificity further with wet lab testing the following Known clinical samples were analyzed using the *Geno Sen's*[®] Dengue Real Time PCR RG kit on ROTOR GENE 3000/6000 machine. The extraction was carried out with the *Geno Sen's*[®] Viral RNA Extraction Mini Kit — for RNA purification (Cat . No. 98001)

The run was carried out with the known set of standards in order to quantitate the Dengue RNA Gene.

Sample Type	Serum	Plasma	CSF
High +ve's	7	4	0
Medium +ve's	13	6	3
Low +ve's	11	5	3
Extremely low +ve's	4	2	0
Negative samples.	4	4	5
	39	21	11

All the above samples were correctly identified by the *Geno Sen's*[®] Dengue Real Time PCR RG kit & all the 6 extremely low samples were accurately detected by the *Geno Sen's*[®] Dengue Real Time PCR RG kit & exhibited copies around 85 copies /ml or less than 85 copies/ml.

Further studies are underway on this aspect.

12. Warranty::

Products are guaranteed to confirm to the quality and content indicated on each vial and external labels during their shelf life. Genome Diagnostics Pvt. Ltd. obligation and purchaser's rights under the warranty are limited to the replacement by Genome Diagnostics Pvt. Ltd of any product that is shown defective in fabrication, and that must be returned to Genome Diagnostics Pvt. Ltd Freight prepaid, or at Genome Diagnostics Pvt. Ltd. option, replacement of the purchasing price. Any complaint on damaged goods during transport must be directed to the handling or transport agent.

In Vitro Diagnostic Medical device, as per the Directive 98/79/EC specifications. This product must be used by qualified professionals only. It is the responsibility of the user to ascertain that a given product is adequate for a given application. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits our responsibility to the replacement of the product. No other warranties, of any kind, express or implied, including without limitation, implicit warranties of commercialization ability or adequacy for a given purpose, are provided by Genome Diagnostics Pvt. Ltd. Genome Diagnostics Pvt. Ltd will not be responsible for any direct, indirect, consequential or incidental damage resulting of the use, misuses, results of the use or inability to use any product.

13. Limitations of product use:

- a.) All reagents may exclusively be used for *in vitro* diagnostics.
- b.) The product is to be used by personnel specially instructed and trained in for the *in-vitro* diagnostics procedures only.
- c.) It is important to pipet the indicated quantities, and mix well after each reagent addition. Check pipettes regularly.
- d.) Instructions must be followed Correctly in order to obtain correct results. If the user has any questions, please contact our Technical Dept. (<u>dharam@vsnl.com</u> or at pbpl@vsnl.net).
- e.) This test has been validated for use with the reagents provided in the kit. The use of other Reagents or methods, or the use of equipment not fulfilling the specifications, may render equivocal results. User is responsible for any modifications done in any of the indicated parameters. Compliance with the kit protocol is required.
- f.) Detection of Viral RNA depends on the number of Viruses present in the sample, and can be affected by sample collection methods, patient-related factors (e.g. age, symptoms), or for infection stage and sample size.
- g.) False negative results may be obtained due to polymerase inhibition. It is recommended to perform control reactions to distinguish between inhibition and true negatives. This is achieved by the inhibition Control included in the kit.
- h.) Cross contamination between samples and exogenous DNA can only be avoided by following good laboratory practice. Instructions in this document must be strictly followed.
- i.) Attention should be paid to expiration dates printed on the kit box and labels of all components. Do not use expired components.

If you have any further questions or problems, please contact our technical support at dharam@vsnl.com OR pbpl@vsnl.net.

14. Publication and reference;

i) Evaluation of four commercial real-time RT-PCR kits for the detection of dengue viruses in clinical samples.

Najioullah F¹, <u>Viron F</u>, <u>Césaire R</u>. Received: 24 December 2013 Accepted: 3 September 2014 Published: 15 September 2014

ii) A CLINICO-MICROBIOLOGICAL STUDY OF DENGUE FEVER CASES IN A

TERTIARY CARE CENTRE OF NAVI MUMBAI

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Received for publication: September 11, 2013; **Revised**: September 27, 2013; **Accepted**: October 17, 2013

iii) Imported cases of dengue fever in Russia during 2010–2013

Author links open the overlay panel. Numbers correspond to the affiliation list which can be exposed by using the show more link.

<u>El Sergeeva * sergeeva.biopalette@gmail.com, VA Ternovoi, EV Chausov, SA Berillo, OK Demina, AN Shikov, IV Plasunova, M Ju Kartashov, AP Agafonov</u>

 iv) Emergence of new lineage of Dengue virus 3 (genotype III) in Lucknow, India Authors: Sanjeev Kumar Tripathi1, Prashant Gupta2,*, Vineeta Khare3, Animesh Chatterjee1, Rashmi Kumar4, Mohammed Yahiya Khan5, Tapan N Dhole1 PMC ID: 3577557 Journal: Iranian Journal of Microbiology Publisher: Tehran University of Medical Sciences License: This is an open-access article distributed under the terms of the

v) Current Global Status of Dengue Diagnostics

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Article Information

DOI: 10.9734/JABB/2015/14505 *Editor(s):* (1) Ram Shanmugam, Professor, School of Health Administration, Texas State University, 601 University Drive, San Marcos, TX 78666, USA. *Review Article* ABSTRACT

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 Hugo Rezende Henriques, Departament of Parasitology, University of São Paulo, Brazil. Complete Peer review History:

15. LIST OF GENO-SEN'S RANGE OF REAL TIME PCR KITS

S.NO.	PRODUCT
1	HIV-1 RG quantitative Real time PCR kit.

IBV RG quantitative Real time PCR kit. ICV RG quantitative Real time PCR kit.
ICV RG quantitative Real time PCR kit.
ICV Genotyping 1/2/3/4 RG qualitative Real time PCR kit.
IEV RG quantitative Real time PCR kit.
AV RG quantitative Real time PCR kit.
EV RG quantitative Real time PCR kit.
NTEROVIRUS RG quantitative Real time PCR kit.
DENGUE RG quantitative Real time PCR KIT
ISV 1 & 2 RG quantitative Real time PCR kit.
CMV RG quantitative Real time PCR kit.
lanta Virus RG quantitative Real time PCR kit.
leasles Virus RG quantitative Real time PCR kit.
Vest Nile Virus RG quantitative Real time PCR kit.
I5 N1 (Bird Flu) RG quantitative Real time PCR kit.
Chikungunya RG quantitative Real time PCR kit.
TV RG quantitative Real time PCR kit.
ARS RG quantitative Real time PCR kit.
C/BK Virus RG quantitative Real time PCR kit.
ITb Complex RG quantitative Real time PCR kit.
ITb Complex /MOTT RG quanlitative Real time PCR kit.
Chlamydia pneumonia RG quantitative Real time PCR kit.
Streptococcous pneumonia RG quantitative Real time PCR kit.
I. Meningitis RG quantitative Real time PCR kit.
I. Influenza RG quantitative Real time PCR kit.

26	Leprosy RG quantitative Real time PCR kit.
27	Helicobacter Pylori RG quantitative Real time PCR kit.
28	Scrub Typhus RG quantitative Real time PCR kit.
29	B. Pseudomalie RG quantitative Real time PCR kit.
30	Filaria RG quantitative Real time PCR kit.
31	Leptospira(pathogenic) RG quantitative Real time PCR kit.
32	CCL3-L1 RG quantitative Real time PCR kit.
33	Malaria (P. Vivax) RG quantitative Real time PCR kit.
34	Bcr/abl Major RG quantitative Real time PCR kit.
35	Bcr/abl Minor RG quantitative Real time PCR kit.
36	PML/RARA RG quantitative Real time PCR kit.
37	RARA/PML RG quantitative Real time PCR kit.
38	GAPDH RG quantitative Real time PCR kit.
39	β-Actin RG quantitative Real time PCR kit.
40	β -Globin RG quantitative Real time PCR kit.
41	Abl gene RG quantitative Real time PCR kit.
42	Rabies RG quantitative Real time PCR kit.
43	Factor V Leiden detection RG Real time PCR kit.

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