# Analysis of negative-strand RNA viruses by RT-qPCR: Rift Valley Fever virus and Toscana virus

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**Running Title :** RT-qPCR analysis of negative-strand RNA Viruses

## Abstract

RT-qPCR allows the detection of viruses and the monitoring of viral replication. This technique was extensively employed during the SARS-CoV2 pandemic, where it demonstrated its efficiency and robustness. Here we describe the analysis of Rift Valley fever and Toscana viruses infection over time, achieved through the RT-qPCR quantification of the viral genome. We further elaborate on the method to discriminate between genomic and antigenomic viral RNA by using primers specific for each strand during the reverse transcription step.

**Key words :** RT-qPCR, Rift Valley fever virus, Toscana virus, bunyavirus, detection method, negative-strand RNA virus

## 1. Introduction

Viruses are constantly emerging, and some can spread worldwide, as illustrated by the case of SARS-CoV-2. Over the past decade, we have observed a large increase in the emergence of neurotropic arboviruses (for “arthropod-borne viruses”), particularly flaviviruses such as West Nile virus (WNV), Usutu virus (USUV), or tick-borne encephalitis virus (TBEV), especially in Europe [1, 2]. Other neurotropic viruses of concern belong to the phenuivirus family, which has a wide geographical distribution. This family encompasses over 100 identified members, including Rift Valley fever virus

(RVFV), Dabie virus (DABV), and Toscana virus (TOSV). These viruses require special monitoring as they can cause serious symptoms in human and in livestock, notably for RVFV. The World Health organization (WHO) lists RVFV among its priority diseases highlighting its high epidemic potential and the urgent need for the development of medical treatments [3].

These threats to public health underscore the critical need for rapid, accurate laboratory techniques to detect, monitor, and study these different viruses. Among these methods, the reference diagnostic technique used during the COVID-19 pandemic was real-time or quantitative reverse transcription polymerase chain reaction (RT-qPCR) [4]. This technique is applicable not only for SARS-CoV-2 but also for many viruses, as evidenced during the Ebola (EBOV) and Zika (ZIKV) epidemics, for example [5, 6]. RT-qPCR has proven to be relatively easy to set up in a laboratory but can also be implemented in the field, particularly in rural endemic areas. In such cases, specific conditions for diagnosis or monitoring of infection must be established to prevent cross-contamination [7]. Moreover, technicians can be readily trained to perform RT-qPCR in either a diagnostic or a research laboratory. Finally, this technique, based on the detection of pathogen-specific genetic material [8], allows the identification of infection without the need for antibody development and with considerably higher sensitivity than immunoassays.

Here, we describe the potency of this tool for the early detection of viruses, the study of their biology and the prevention of their spread. We illustrate the sensitivity, specificity, and robustness of RT-qPCR in detecting RVFV and TOSV, the latter presented here as another instance of negative-strand virus detection, which is simpler to handle because it is a biosafety level (BSL)-2 pathogen compared to RVFV which is BSL-3.

### 1.1 The arthropod-borne viruses RVFV and TOSV

RVFV and TOSV are arboviruses belonging to the genus *Phlebovirus*, in the *Phenuiviridae* family and the *Bunyavirales* order. RVFV is a zoonotic disease transmitted by mosquitoes mainly from *Aedes* and *Culex* genera. During its life cycle, RVFV can infect a wide range of hosts, notably domestic livestock such as sheep, goats, and cattle causing high rates of neonatal mortality and abortion with serious

economic consequences for the affected countries [9]. Humans can be infected by mosquito bites or direct contact with the tissues or blood of infected animals, leading to epidemics. Symptoms in humans range from febrile illness to neurological (<1% of patients), ocular damage (<0,5 to 2%), and haemorrhagic fever (<1%) [10, 11]. The last RVFV epidemic occurred in Niger in 2016, and due to the variable and non-specific symptoms, clinical diagnosis is difficult to establish.

TOSV is transmitted by phlebotomine sandflies, mainly of the *Phlebotomus perniciosus* and *perfiliewi* genera [12]. The virus circulates in all Mediterranean basin countries, and some imported cases have been reported in North America and Australia [13–15]. TOSV is essentially asymptomatic but can cause mild symptoms and, in severe cases, meningitis or encephalitis. Due to the febrile nature of the disease and limited access to accurate diagnostic methods, the number of infected people is largely underestimated.

Similar to other phleboviruses, RVFV and TOSV particles are enveloped and roughly spherical, with a single-stranded, tripartite RNA genome. The three RNA segments consist of L (for large), M (for medium), and S (for small) segments. The L and M segments are of negative-sense polarity and encode the RNA-dependent RNA polymerase (RdRp) L and a polypeptide precursor M, respectively. Proteolytic cleavages of the precursor M results in the non-structural protein NSm and the two surface glycoproteins, Gn and Gc, which are involved in the entry of virions into host cells (Fig. 1) [16]. The S segment is of ambisense polarity and encodes the nucleoprotein N and the non-structural protein NSs which is known to be a major viral virulence factor of phleboviruses [17]. Interestingly, the N protein is translated directly from an mRNA (N mRNA) transcribed from the viral genomic RNA (genomic vRNA), while NSs is translated from an mRNA (NSs mRNA) transcribed from the viral antigenomic RNA (antigenomic vRNA), forming a replicative intermediate **(**Fig. 2**)**. This negative RNA polarity implies that the bunyavirus carry out replication in two steps: first, the *de novo* synthesis of a positive-polarity intermediate RNA, and second, the use of this as a template to synthetize the new generation of negative-polarity genomic vRNA [18, 19].

### 1.2 RT-qPCR detection method

Reverse transcription (RT) is required to convert vRNA into complementary DNA (cDNA) which serves as the template for qPCR amplification. Consequently, qPCR enables early detection and monitoring of the first stages of viral replication. It can also be employed to diagnose specific mutations or screen antiviral molecules, owing to its speed, sensitivity, and specificity. RT can be performed to reverse transcribe either (i) all RNAs present in a cell or (ii) only specific RNAs. In the former case, in addition to all cellular mRNAs, the RT step will permit the amplification of the whole viral genome (genomic and antigenomic) and messengers. This total cDNA also allows the quantification of other genes of interest and housekeeping genes in the same sample if required. It is performed using a mixture of oligodT and random hexamers primers (*see* **Note 1**). In the latter case, RT is performed with specific primers in order to select the RNA to be reverse transcribed. Here, in addition to a whole RNA amplification, we designed specific primers for the RT to discriminate between the genomic and antigenomic strands, in order to specifically quantify their synthesis during viral infection (Fig. 2).

In the next step, qPCR is performed using primers that are specific for the region of interest (Fig. 3) and fluorescent compounds that bind to the double-strand DNA, resulting in a fluorescent signal proportional to the amount of DNA synthesized in each PCR cycle [22]. Two main RT-qPCR techniques exist to quantify DNA: (i) SYBR Green fluorescent dyes and (ii) TaqMan probes [23]. In this case, SYBR Green is as specific and robust as probes, but much more cost-effective and flexible. To normalise the samples, we selected two housekeeping (HK) genes: ribosomal protein L22 (RPL22) and β-glucuronidase (GusB), and quantified the viral RNA using the $2^{(-\Delta CT)}$ method [24]. In the case of the RT with primers specific for the two viral strands, specific primers for the housekeeping genes were also designed and added to the mix. The final calculation considers the Ct of the virus over the mean Ct values of the two house-keeping genes. The following results illustrate viral replication over time in A549 cells infected with either TOSV or RVFV (MOI 2). Here we quantified the RNA encoding the N-protein, discriminating between the genomic and antigenomic strands, and compared it with the total amount including genomic strand, antigenomic strand, and mRNA (*see* **Note 2**).

## 2. Materials

### 2.1 Viral infection

1. Dulbecco's Minimum Essential Medium (DMEM).
2. A549 cells : cultured in DMEM, supplemented with 10 % fetal bovine serum (FBS).
3. Phosphate buffered saline (PBS) 1x.
4. Trypsin-EDTA (0.25%).
5. Any standard 12-well plate.
6. Haemocytometer or cell counter.
7. 1.5 mL Eppendorf tubes.

### 2.2 RNA extraction

1. TRIzol reagent.
2. Chloroform.
3. RLT cell lysis buffer contained in RNeasy Plus kit (QIAGEN) (*see* **Notes 3 and 4**).
4. Ethanol 96-100%.
5. Any RNAse DNAse free 1.5 mL tubes.
6. Any refrigerated 1.5 mL tube centrifuge able to centrifuge at 12 000 g.
7. Spectrophotometer able to quantify small amounts of RNA.

### 2.3 Retro-transcription step

1. PrimeScript™ RT reagent Kit (Takara Bio, Inc.) or any retrotransciption kit containing ‘random primers’ (*see* **Note 5**).
2. 8-tube strips with domed caps attached, 0.2 mL.
3. 1.5mL DNase RNase-free tubes.

4. RNase-free water.
5. Specific RT primers targeting either the genomic or antigenomic S segment of TOSV/RVFV.
6. Specific RT primers targeting the HK gene of interest (Rpl22 and GusB).
7. Any type of thermal cycler for 0.2 mL tubes.

### 2.4 Quantitative PCR

1. PrimeScript™ RT reagent Kit TB Green® Premix Ex Taq™ (Takara Bio,Inc) or any intercalator-based real-time PCR kit using fluorescent dye (*see* **Note 6**).
2. RNase-free water.
3. Primers targeting your gene of interest (in our case, Rpl22, GusB, TOSV, and RVFV).
4. 96-well plate cooling block.
5. Any 96-well plates, skirted and low profile adapted to the real-time PCR System instrument.
6. Optical caps, 8x strips for 96-well plates.
7. Real-time PCR system instrument (*see* **Note 7**).
8. Any refrigerated plate centrifuge.
9. 1.5mL DNase RNase-free tubes.

## 3. Methods

### 3.1 Viral infection

1. 24 hours before infection: seed $2.5x10^5$ A549 cells in 1mL DMEM 10% FBS in a 12-well plate and maintain cells at 37°C and 5% CO2 (*see* **Note 8**).
2. Infect cells at MOI 2 in a total volume of 500 µL of DMEM 0% FBS per well for 1h.
3. After 1h, remove the supernatant and rinse the cells with 1 mL of 1x PBS before adding 1 mL DMEM 2% FBS.
4. Harvest infected cells at desired timepoints (see **Notes 9 and 10**).

5. Aspirate the cell culture medium and add 500 µL of TRIzol reagent to detach and harvest the cells.
6. Homogenise and harvest the cells using a P1000 pipette. Medium may be sticky, indicating DNA release.
7. Transfer the lysate into a 1.5 mL Eppendorf tube.

**3.2 RNA extraction (*see* Note 11)**

1. Add 180 µL of chloroform in each tube.
2. Mix by pipetting up and down five times.
3. Incubate for 2 min at room temperature.
4. Centrifuge at 12000 g, 5 min, 4 °C.
5. Collect the aqueous phase (approximately 450 µL) in a 1.5mL Eppendorf tube and place on ice.
6. Dispatch 100 µL of this aqueous phase into a 1.5 mL tube (*see* **Note 12**).
7. Add 350 µL of RLT buffer and 250 µL 96-100 % ethanol to the 100 µL of aqueous phase.
8. Mix by pipetting up and down.
9. Load the mixture onto the RNA column.
10. Centrifuge at 8000 g for 15 sec at room temperature.
11. Discard the eluate.
12. Repeat the loading procedure (100 µL aqueous phase +350 µL RLT buffer + 250 µL ethanol 96-100%) on the same column until the end of the aqueous phase sample volume.
13. Wash twice.
14. Add 500 µL RPE buffer.
15. Incubate for 1 min.
16. Centrifuge at 8000g for 15 sec at room temperature.
17. After the second wash, centrifuge at 12000 g, 1 min at room temperature to dry the column.
18. Elute the RNA with 30 µL of RNA free water onto a 1.5 mL RNAse DNAse free tube.
19. Incubate 1 min.

20. Centrifuge at 8000 g for 1 min.
21. Samples can be frozen at -20°C.

### 3.3 Retro-transcription step

1. See Table 1 for the different RT primers sequences.
2. Prepare the RT reaction mixtures on ice (Tables 2 and 3).
3. Incubate the reaction mixtures at the following conditions using the thermal cycler:
    - 37°C or 42°C 15 min (*see* **Note 13**) (reverse transcription).
    - 85°C 5 sec (inactivation of reverse transcriptase with heat treatment).
    - 4°C ∞.
4. After the incubation time, keep samples on ice.

### 3.4 Quantitative PCR

1. qPCR was realized using 20 ng of cDNA for quantification ot the two HK genes and 2 ng for quantification of TOSV or RVFV RNA (*see* **Note 14**).
2. In a qPCR 96-well plate, dispense 2 µL of the appropriate dilution of cDNA sample depending on the gene (*see* **Note 15**).
3. See Table 4 for the different qPCR primers sequences.
4. Prepare the qPCR mix on ice (Table 5).
5. Add 18 µL of qPCR mixture in each well (*see* **Note 16**).
6. Seal each p96 well with plugs to avoid evaporation.
7. Centrifuge the plate for 30 sec at 900 g at 4°C (*see* **Note 17**).
8. On a real-time PCR system instrument (here AriaMx, Agilent), program the following thermal profile (Fig. 4) and run the reaction.
9. Analyse the results by calculating the $2^{(-\Delta CT)}$, *i.e.* Ct of the virus over the mean Ct values of the two house-keeping genes (Fig. 5). The amount of genomic RNA is quite comparable to total RNA, due to the abundance of negative S viral RNA in particles and infected cells. In contrast,

the amount of antigenomic strand remains low and reaches a plateau 24h post-infection. This illustrates that one positive strand is used as a template to synthesise several viral genomic RNA and may also correlate with infection of 100% of the cells. The presence of genomic RNA segments in the intracellular lysate has already been described for RVFV and Uukuniemi virus (UUKV), another bunyavirus closely related to phleboviruses [25, 26]. To conclude, our results show that it is possible to distinguish negative and positive strands by RT-qPCR during bunyavirus infection to follow viral replication (*see* **Notes 18 and 19**). These experiments could be extended to other negative RNA strand viruses.

## 4. Notes

1. OligodT are used for better amplification of the signal for the HK genes. Indeed, viral mRNA is capped but not polyadenylated, oligodT are unnecessary.
2. Standard curves have been run upstream for each primers pairs, to calculate their efficiencies with an amount of 20 or 2 ng of RNA. Primers pairs were selected if efficiency was comprised between 1.9 and 2.1.
3. TRIzol/chloroform and RNeasy Plus kit (QIAGEN) containing RLT cell lysis buffer have been optimized for RVFV RNA extraction and previously tested to inactivate it in BSL-3 conditions but any other RNA extraction kits and adapted cell lysis buffer can be used.
4. NucleoSpin RNA, Mini kit for RNA purification (Macherey-Nagel) containing RA1 cell lysis buffer have been optimized for the following TOSV RNA extraction and previously tested to inactivate it in BSL-2, but any other RNA extraction kits and adapted cell lysis buffer can be used.
5. In the following protocol, PrimeScript RT reagent kit (Takara Bio, Inc) is the reference kit but the volumes may be adjusted depending on the retrotranscription kit chosen and the manufacturer's recommendations.
6. In the following protocol, PrimeScript™ RT reagent Kit TB Green® Premix Ex Taq™ (Takara Bio, Inc) is the reference kit for real-time PCR. Conditions have been optimized for RVFV and TOSV with our thermocycler (Agilent, AriaMx) but any other kits can be used.

7. qPCR quantifications were performed using the AriaMx Real-time PCR System instrument (Agilent Technologies) and adaptated Aria-Mx 96-well plates (Agilent Technologies) with optical caps, 8x strips for use with AriaMx 96-well plates (Agilent Technologies).
8. Seed an extra well of cells to determine the amount of virus required to achieve an appropriate multiplicity of infection (MOI). The day of infection :

   - Rinse cells with 1mL DMEM 0% FBS.

   - Count the cells in the extra well to adjust the number of viral infectious particles : remove the media, rinse the cells with 1 mL 1x PBS, clear the well and add 200 µL of trypsin, let cells detach.

   - Resuspend by adding 500 µL of DMEM 0% FBS and count the cells using a haemocytometer.
9. Cells were harvested 2h, 9h, 12h and 24h post-infection for TOSV and 2h, 8h and 24h post-infection for RVFV.
10. This protocol was tested for the inactivation of RVFV in BSL-3 conditions. Another protocol was performed for TOSV which is validated in a BSL-2 laboratory with RA1 cell lysis buffer contained in NucleoSpin RNA, Mini kit for RNA purification (Macherey-Nagel). To apply this latter method:

    - Prepare cell lysis buffer mix (350 µL RA1 buffer + 7 µL DTT 1M per well).

    - Aspirate cell-culture medium and add an equal volume of PBS in order to wash the cells. Avoid incomplete removal of the cell culture medium in order to allow full lysis activity of the buffer.

    - Immediately add the lysis buffer mix to the cell culture well.

    - Transfer the lysate into a 1.5 mL Eppendorf tube.

    Two 12-well plates can be pooled together in order to increase the RNA concentration, in this case, add the lysis buffer to the first well, collect its contents and add the mixture directly to the second well, leaving a final volume of 357 µL.
11. For TOSV, RNA extractions were performed according to the manufacturer's instructions for NucleoSpin RNA, Mini kit for RNA purification (Macherey-Nagel) (*see* **Note 11.1**). RNA concentration was quantified using Nanodrop 2000 (thermofisher) (*see* **Note 11.2**) and RNA was diluted to a final concentration of 500 ng in a total volume of 6.5 µL of RNase-free water.

11.1 Elute the RNA in 50µL RNase-free H2O, (supplied) and centrifuge at 11000 g for 1 min.

11.2 Check the different measurement reports. The A260/A280 ratio can indicate protein contamination in your sample. The A260/A280 ratio should be between 1,8 and 2,0 in a purified DNA sample and 2.0 is generally accepted for purified RNA. Lower values indicate protein contamination, while higher values indicate RNA contamination. In addition, a low 260/280 ratio may also indicate the presence of phenol, an additive used in some DNA purification methods. In addition to protein contamination, certain contaminants such as phenol, EDTA, guanidine thiocyanate, Triton X-100 or carbohydrates can cause a relative increase in absorbance at 230 nm compared to 260 nm (decreased A260/A230 ratio). The A260/A230 is expected to be between 2.0 and 2.2 [27].

12. If the volume of 100 µL is incomplete, add water to reach it.
13. If using a gene-specific primer, perform the reverse transcription at 42°C for 15 min instead of 37°C for 15 min with random primers.
14. Dilute samples to a final concentration of 10 ng/µL by adding 40 µL of RNase-free water for quantification of HK genes. Perform another dilution at 1 ng/µL by adding 2 µL of 10 ng/µL mRNA with 18 µL of RNase-free water for viral RNA quantification. Diluted samples at 10 ng/µL and 1 ng/µL are stored at -20°C and undiluted samples at -80°C.
15. Change tips at each step to avoid cross contamination and keep your plate on the 96-well plate cooling block during the loading.
16. qPCR runs are performed in duplicate and a point containing RNase-free water is also performed in order to make sure that there is no contamination. Duplicates are considered reliable if they differ by less than 0.5 Ct.
17. Clean the plate with ethanol before running the reaction on the real-time PCR System instrument.
18. Infectious and defective virus particles cannot be distinguished because it is impossible to determine the number of each segment in the viral particle simply with RT-qPCR.
19. This RT-qPCR method is also applicable to purified virus or supernatant of infected cells with an appropriate extraction kit (*e.g.* QIAGEN, QIAamp Viral RNA kit) but without the housekeeping genes normalization.

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**Figure legends**

**Figure 1: Genomic organisation of phleboviruses such as RVFV and TOSV**

A typical phleboviral particle is represented on the left, containing the three segments: large (L), medium (M), and small (S) of the viral genome. The lengths in nucleotides are indicated at the top of each genomic segment. The viral M segment encodes a polypeptide precursor, whose proteolytic cleavage results in the nonstructural protein NSm and the two surface glycoproteins, Gn and Gc. The S segment has an ambisense polarity and encodes the nucleoprotein N and the non-structural protein NSs [20, 21].

**Figure 2: Genomic organisation of the phlebovirus S segment and hybridization sites of specific retrotranscription primers.**

The two specific primers used for RT are represented for each virus, RVFV or TOSV. "RT_S-" (in blue) permits the reverse transcription of the genomic vRNA (negative polarity) whereas "RT_S+" (in pink) permits the reverse transcription of the antigenomic vRNA (positive polarity).
(ss = single strand).

**Figure 4: Thermal profile of the quantitative PCR on Agilent Mx thermocycler.**

**Figure 3: Hybridization sites of qPCR primers on S segment cDNA.**

Forward (F) and reverse (R) primers used during qPCR are represented in green for each virus.
(ds = double strand).

**Figure 5: Quantification of mRNA from TOSV (A) and RVFV (B) S segments.**

A549 cells were infected at MOI 2. At indicated times, cells were harvested and then mRNA was extracted and retrotranscribed into cDNA using specific or random primers. Black lines indicate the quantification of viral and messenger RNA from retrotranscription using random primers, *i.e.*, it shows the total quantity of TOSV S and RVFV S segments (genomic, antigenomic, and N mRNA). Pink lines indicate the quantification of antigenomic vRNA from retrotranscription using the specific primer RT_S+ in order to quantify the S positive strand. Blue lines indicate the quantification of genomic vRNA from retrotranscription using the specific primer RT_S- in order to quantify the S negative strand. GusB and Rpl22 were used as housekeeping genes to normalise expression.

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**Table 1. RT primers sequences**

| Name | Primer sequence |
|---|---|
| **RT_hGUSB** | TCAGTAGCCACTTTCATGCCAACTC |
| **RT_hRPL22** | CAAACAGTCACTACCTTCAGCCC |
| **RT_TOSV_S-** | ACACAGAGATTCCCGTGTATTAATCA |
| **RT_TOSV_S+** | ACCTCCCGTATCGCTAAAC |
| **RT_RVFV_S-** | ACACAAAGCTCCCTAGAGATACAAAC |
| **RT_RVFV_S+** | ACACAAAGACCCCCTAGTGC |

**Table 2. Reverse transcription mix with random primers**

| Reagent | Volume | Final concentration |
|---|---|---|
| 5x PrimeScript buffer (for real time) | 2 µL | 1x |
| PrimeScript RT Enzyme Mix 1 | 0.5 µL | |
| Oligo dT Primer (50 µM) | 0.5 µL | 2.5 µM |
| Random 6 mers (100 µM) | 0.5 µL | 5 µM |
| Total RNA | 6.5 µL | 50 ng/µL |
| **Total** | **10µL** | |

**Table 3. Reverse transcription mix with specific primers**

| Reagent | Volume | Final concentration |
|---|---|---|
| 5x PrimeScript buffer (for real time) | 2 µL | 1x |
| PrimeScript RT Enzyme Mix 1 | 0.5 µL | |
| Specific RT primer (GusB) (10 µM) | 0.5 µL | 0.5 µM |
| Specific RT primer (Rpl22) (10 µM) | 0.5 µL | 0.5 µM |
| Specific RT primer (TOSV/RVFV) (10µM) | 0.5 µL | 0.5 µM |
| Total RNA | 6.5 µL | 50 ng/µL |
| **Total** | **10 µL** | |

**Table 4. qPCR primers sequences:**

| Name | Primer sequence |
|---|---|
| **qPCR_hGUSB** | Forward: GATTGCCAATGAAACCAGGTATC<br>Reverse: ACACGCAGGTGGTATCAGTCTT |
| **qPCR_hRPL22** | Forward: TCGCTCACCTCCCTTTCTAA<br>Reverse: TCACGGTGATCTTGCTCTTG |
| **qPCR_TOSV** | Forward: AGGTGGCTGCTACGTTTGAA<br>Reverse: CTCAACCCCATGGCAGCTTA |
| **qPCR_RVFV** | Forward: ATGAGAGCCTCCACAGTTGC<br>Reverse: CTGGCTCTAACTCGTGGCAA |

**Table 5. qPCR mix**

| Reagent | Volume | Final concentration |
|---|---|---|
| TB Green Premix Ex Taq II (2x) | 10 µL | 1x |
| qPCR Forward primer (10 µM) | 0.2 µL | 0.1 µM |
| qPCR Reverse primer (10 µM) | 0.2 µL | 0.1 µM |
| RNase DNase free water | 7.6 µL | |
| **Total** | **18 µL** | |

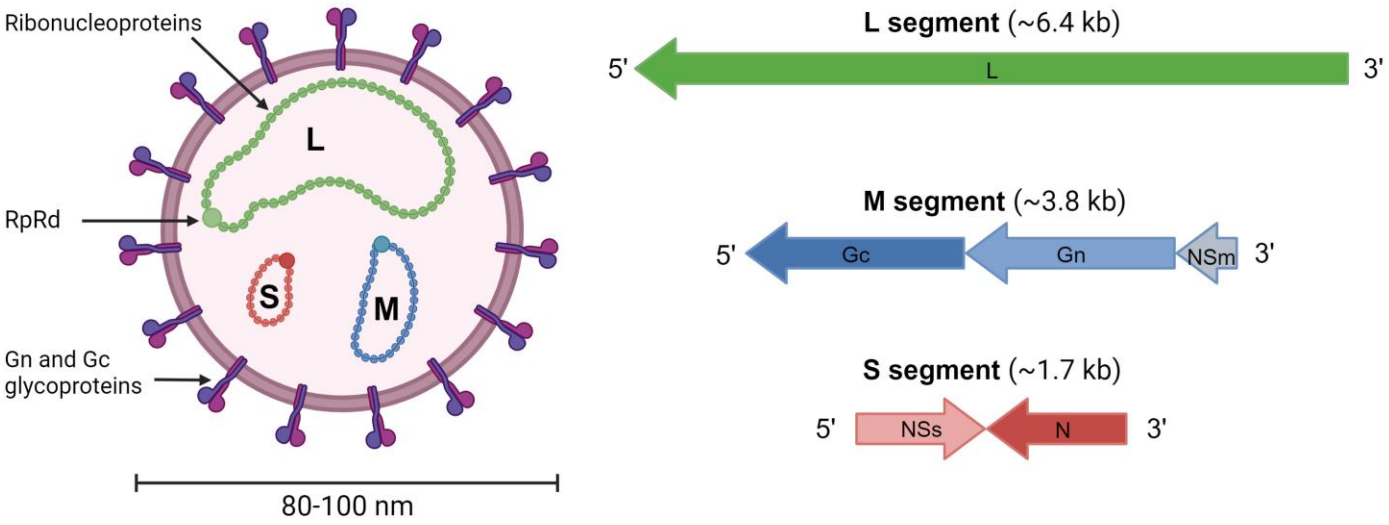
Ribonucleoproteins
L
RpRd
S
M
Gn and Gc
glycoproteins
80-100 nm
L segment (~6.4 kb)
5'
L
3'
M segment (~3.8 kb)
5'
Gc
Gn
NSm
3'
S segment (~1.7 kb)
5'
NSs
N
3'

Fig. 1

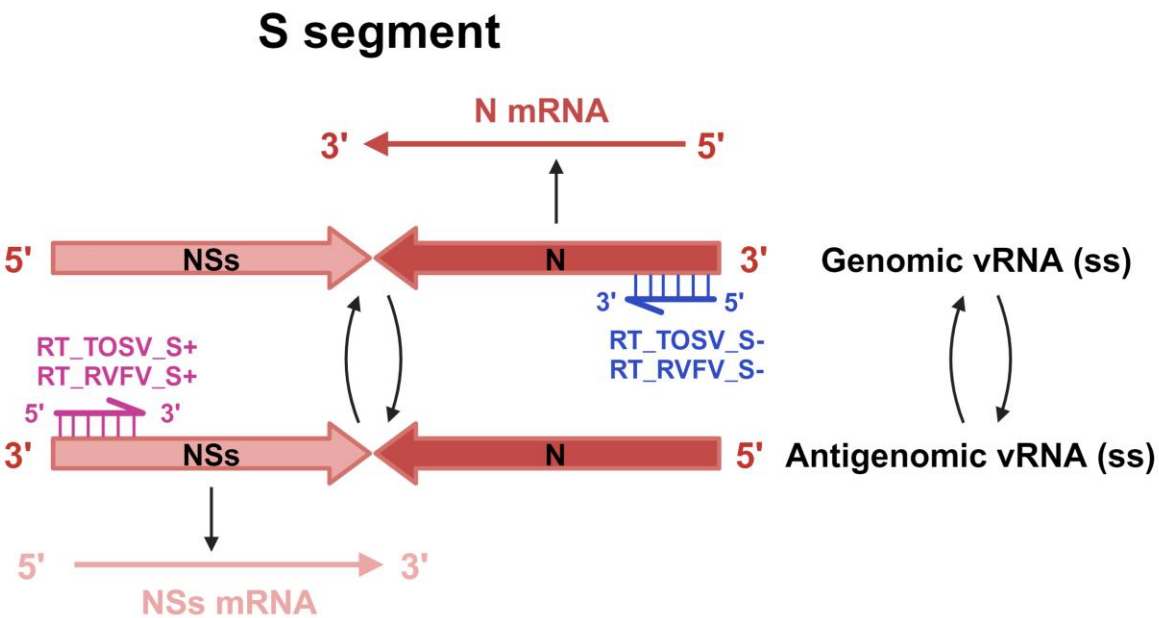
S segment
N mRNA
3'
5'
5'
NSs
N
3'
Genomic vRNA (ss)
3'
5'
RT_TOSV_S-
RT_RVFV_S-
RT_TOSV_S+
RT_RVFV_S+
5'
3'
3'
NSs
N
5'
Antigenomic vRNA (ss)
5'
3'
NSs mRNA

Fig. 2

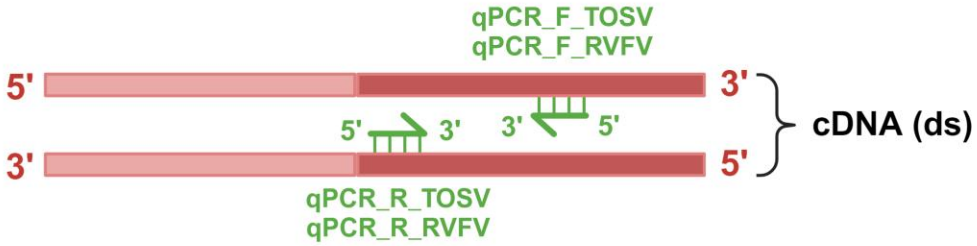
qPCR_F_TOSV
qPCR_F_RVFV
5'
3'
5'
3'
3'
5'
3'
5'
cDNA (ds)
qPCR_R_TOSV
qPCR_R_RVFV

Fig. 3

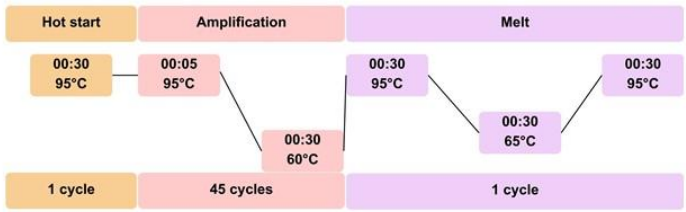
Hot start
Amplification
Melt
00:30
95°C
00:05
95°C
00:30
60°C
00:30
95°C
00:30
65°C
00:30
95°C
1 cycle
45 cycles
1 cycle

Fig. 4

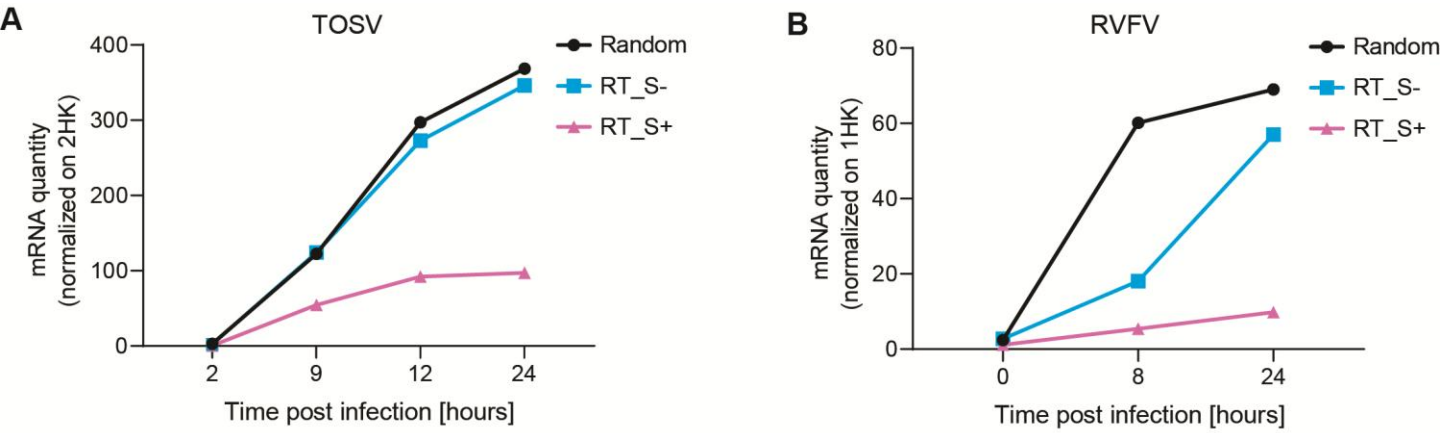
A
TOSV
400
300
200
100
0
mRNA quantity
(normalized on 2HK)
2
9
12
24
Time post infection [hours]
Random
RT_S-
RT_S+
B
RVFV
80
60
40
20
0
mRNA quantity
(normalized on 1HK)
0
8
24
Time post infection [hours]
Random
RT_S-
RT_S+

Fig. 5