Validation of SYBR Green based quantification assay for the detection of human Torque Teno virus titers from plasma

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Abstract

Background: Quantification of titers of ubiquitous viruses such as Torque teno virus (TTV) that do not cause clinical symptoms might be helpful in assessing the immune status of an individual. We hereby describe the validation of a SYBR Green-based TTV quantification method for plasma samples.

Methods: Plasmids with TTV specific inserts were used for preparing standards and absolute quantification of TTV was performed using SYBR Green methodology. The method was assessed for its accuracy and precision (intra and inter-day) on four non-consecutive days. TTV was also quantified from plasma samples of 20 healthy volunteers and from 30 hematopoietic stem cell transplant (HSCT) recipients.

Results: The assay was specific and showed satisfactory efficiency (82.2%, R²=0.99) with the limit of quantification defined as 100 copies per reaction. The assay had good precision (inter and intra-day coefficient of variation in cycle threshold (CT) < 4%) and accuracy (100 ± 10%) in the range of 100 to 1010 copies/reaction. We found TTV loads ranging from 2.5 – 4.07 log copies/mL of plasma with CT (mean ± SD) of 33.8 ± 1.77 in healthy individuals and 2.06 – 8.49 log copies/mL of plasma with CT (mean ± SD) of 24.3 ± 1.04 in HSCT recipients.

Conclusion: SYBR Green-based q-PCR assay combines simplicity with satisfactory sensitivity and may be suitable for monitoring the immune status of transplant recipients, where TTV loads over time may serve as a marker for immune reconstitution in human plasma samples.

Keywords: SYBR Green, Real-time PCR, Human torque teno virus
validation of SYBR Green based quantification assay for routine use by using a set of primer pairs targeted for amplifying a well-conserved sequence of ORF-2 [14].

**Results and discussion**

The assay was validated with serial dilutions of standards ranging from 100 to $10^{10}$ copies per reaction. The dual sets of primers used showed satisfactory amplification on four different days with assay efficiencies in the range of 81.8% - 82.9% (slopes of the standard curve $-3.81$ to $-3.85$). The assay was linear in the range of standards used (co-efficient of regression, $R^2$) of 0.99 (Figure 1), which indicates a good correlation between viral copy numbers and cycle threshold ($C_T$) values. The lower limit of quantification was determined as 100 copies/reaction (equivalent to 3000 copies/mL of plasma) with an accuracy of 100 ± 5% and intra and inter-day coefficient of variations below 4% (Table 1). The observed melting curve (Melting temperature $T_M$: 85.01°C ± 0.27 (mean ± SD; Figure 2) that was clearly different from the melting curve of primer-dimers ($T_M$ ranging from 71.1-72.9°C) as well as the purity of the TTV-specific 96 bp amplicon in the samples that contained TTV DNA (Figure 3) confirmed the high specificity of the PCR.

SYBR Green Real-time PCR quantification assay was reproducible with good inter (co-efficient of variation (CV): 0.22 to 1.23%), and intra-day (CV: 0.01 to 3.10%) precision (Table 1). The calculated standard copy numbers were accurate with intra-day and inter-day accuracies in the range of 86.3 to 102.3% and 88.2 to 102.3%, respectively (Table 1). We also observed good reproducibility of the assay when standards were run in triplicates on two different days with intra- and inter-assay precisions of 0.08 to 3.93% and 0.45 to 2.69%, respectively. The intra and inter-day accuracies were 83.8 to 102.1% and 84.3 to 101.3%, respectively (Table 2). Furthermore, our assay yielded similar TTV titers ($\pm$ 10% variation) in positive controls (4.75 log copies/mL and 2.94 log copies/mL) that were kindly provided by Maggi’s group, Pisa, Italy.

In order to check the robustness of the SYBR Green qPCR assay, we measured TTV titers in plasma samples of 20 healthy individuals and 30 HSCT recipients. Variations in TTV loads in terms of log copy numbers of TTV genomes per mL of plasma were found to be in the range of 2.5 – 4.07 log copies/mL (Figure 4A) with a $C_T$ (mean ± SD) of 33.8 ± 1.77 for healthy individuals and 2.06 – 8.49 log copies/mL (Figure 4A) with a $C_T$ (mean ± SD) of 24.3 ± 1.04 for HSCT recipients. In addition, we observed the differences in melting curves for HSCT recipients ($T_M$ ranging from 82.61°C-84.85°C; Figure 4B) which might be due to possible sequence heterogeneity of human TTV strains, with the presence of TTV specific products (96 bp) on 3% agarose gel electrophoresis (Figure 4C).

Several TTV studies using TaqMan chemistry have reported varying levels of sensitivity, ranging from 120 to 1000 copies/mL for different types of clinical specimens [5,14,15,17-22], which may be the result of differences in the primers used. Although sequence heterogeneity in TTV is high with some variants only sharing 50% of nucleotides [23,24], certain conserved regions can be chosen for primer design in order to amplify more than one subtype of TTV [25]. Focosi et al. [5] and

![Figure 1](https://example.com/figure1.png)

**Figure 1** SYBR Green based standard curve from two independent 10 fold serial dilutions of plasmid standards. Standard curve was plotted in the sample plasmid on the x-axis and threshold cycle ($C_T$) on the y-axis. The x-axis represents human TTV in 10-fold dilutions (Log copies) and the y-axis the fluorescence data used for $C_T$ determinations in ΔRn (baseline-corrected normalized fluorescence). Assay was in linear range of Human TTV with $R^2$ values (square of the correlation coefficient) of 0.99.
Maggi et al. [19] used probes directed against the conserved portion of untranslated region (UTR), while others used probes specific for highly conserved region of ORF2 and ORF1 of TTV [14,15,17].

For our qPCR protocol we used the primers described by Moen et al. [14] that differentiate between TTV and TTV-like mini virus (TLMV). Although TaqMan based assays may be somewhat more sensitive, we opted for SYBR Green real-time PCR assay for TTV using two independent standards series

Table 1 SYBR Green real-time PCR assay for TTV using two independent standards series

| Plasmid – standards | Day | C\text{\textsubscript{T}} Mean ± SD (n=2)* | Intra-day precision | Inter-day precision | Intra-day accuracy | Inter-day accuracy |
|---------------------|-----|----------------------------------------|--------------------|-------------------|-------------------|-------------------|
| 10 × 10\textsuperscript{9} | 1   | 4.11 ± 0.09                            | 2.22               | 0.74              | 99.9              | 100.1             |
|                     | 2   | 4.17 ± 0.08                            | 2.03               |                   | 99.8              |                   |
|                     | 3   | 4.11 ± 0.09                            | 2.36               |                   | 99.7              |                   |
|                     | 4   | 4.09 ± 0.02                            | 0.50               |                   | 100.2             |                   |
| 10 × 10\textsuperscript{8} | 1   | 7.14 ± 0.07                            | 1.10               | 0.92              | 102               | 102.3             |
|                     | 2   | 7.24 ± 0.22                            | 3.10               |                   | 101.8             |                   |
|                     | 3   | 7.08 ± 0.06                            | 0.88               |                   | 102.1             |                   |
|                     | 4   | 7.11 ± 0.10                            | 1.53               |                   | 102.3             |                   |
| 10 × 10\textsuperscript{7} | 1   | 11.15 ± 0.05                           | 0.45               | 0.85              | 101.2             | 101.4             |
|                     | 2   | 11.03 ± 0.16                           | 1.49               |                   | 101.6             |                   |
|                     | 3   | 11.26 ± 0.12                           | 1.07               |                   | 100.8             |                   |
|                     | 4   | 11.19 ± 0.06                           | 0.58               |                   | 101.1             |                   |
| 10 × 10\textsuperscript{6} | 1   | 15.21 ± 0.03                           | 0.20               | 0.31              | 99.8              | 100.2             |
|                     | 2   | 15.10 ± 0.02                           | 0.15               |                   | 100.1             |                   |
|                     | 3   | 15.19 ± 0.01                           | 0.11               |                   | 100               |                   |
|                     | 4   | 15.19 ± 0.003                          | 0.02               |                   | 99.9              |                   |
| 10 × 10\textsuperscript{5} | 1   | 19.15 ± 0.009                          | 0.04               | 0.28              | 98.5              | 98.9              |
|                     | 2   | 19.03 ± 0.06                           | 0.35               |                   | 98.8              |                   |
|                     | 3   | 19.14 ± 0.02                           | 0.10               |                   | 98.9              |                   |
|                     | 4   | 19.11 ± 0.02                           | 0.12               |                   | 98.6              |                   |
| 10 × 10\textsuperscript{4} | 1   | 22.89 ± 0.009                          | 0.03               | 0.30              | 97.9              | 97.8              |
|                     | 2   | 22.86 ± 0.09                           | 0.43               |                   | 97.5              |                   |
|                     | 3   | 22.98 ± 0.13                           | 0.57               |                   | 97.9              |                   |
|                     | 4   | 23.00 ± 0.05                           | 0.25               |                   | 96.8              |                   |
| 10 × 10\textsuperscript{3} | 1   | 26.95 ± 0.06                           | 0.24               | 0.22              | 94.2              | 94.3              |
|                     | 2   | 26.87 ± 0.04                           | 0.17               |                   | 93.9              |                   |
|                     | 3   | 26.95 ± 0.005                          | 0.01               |                   | 95.3              |                   |
|                     | 4   | 27.02 ± 0.13                           | 0.50               |                   | 92.9              |                   |
| 10 × 10\textsuperscript{2} | 1   | 30.83 ± 0.16                           | 0.52               | 0.35              | 89.6              | 88.2              |
|                     | 2   | 30.89 ± 0.08                           | 0.26               |                   | 87.2              |                   |
|                     | 3   | 31.08 ± 0.03                           | 0.12               |                   | 88.5              |                   |
|                     | 4   | 30.97 ± 0.13                           | 0.42               |                   | 86.6              |                   |
| 10 × 10\textsuperscript{1} | 1   | 34.29 ± 0.30                           | 0.89               | 0.66              | 86.3              | 92.7              |
|                     | 2   | 33.78 ± 0.57                           | 1.70               |                   | 93.8              |                   |
|                     | 3   | 33.93 ± 0.77                           | 2.29               |                   | 97.8              |                   |
|                     | 4   | 33.87 ± 0.56                           | 1.65               |                   | 92.3              |                   |

*2 independent 10-fold dilution series (n=2) of plasmid standards.

For our qPCR protocol we used the primers described by Moen et al. [14] that differentiate between TTV and TTV-like mini virus (TLMV). Although TaqMan based assays may be somewhat more sensitive, we opted for SYBR Green real-time PCR assay using a primer pair rather than TaqMan technology, which uses two primers and one probe, and is probably more prone to the problem of variable amplification efficacy of strains differing for single nucleotides. Indeed, in our sequencing results for 12 separate clones (Figure 5) from one single patient we
observed sequence heterogeneity of TTV in the region that has been used for TaqMan probe [14]. In general, SYBR Green methodology may be more suitable for viral studies where sequence heterogeneity is high in comparison to TaqMan probe-based assays which require high sequence identity for successful probe binding to avoid frequent variable results [14]. In addition, the assay’s threshold of 3000 copies/mL of plasma may be low enough for monitoring TTV in immune deficient patients.

**Conclusion**

This report describes the validation of a SYBR Green assay for quantification of TTV viral load in human plasma samples. The developed assay was accurate with satisfactory efficiency, reproducibility in the range of
100-10^10 copies/reaction. This simple assay can be used in studies assessing TTV plasma loads as a marker of immune reconstitution. A prospective study is currently ongoing in our institution to validate the correlation of TTV titer and the immune status post HSCT.

**Methods**

**Reagents and consumables**

QIAamp® MinElute virus spin kit for DNA extraction, QIAprep® Spin Miniprep kit for plasmid extraction and QIAquick® PCR purification kit were obtained from Qiagen, Germany. TA cloning kit dual promoter (pCRII) with One Shot TOP10F’ competent cells and ampicillin were obtained from Invitrogen, San Diego, California. DNA Taq polymerase, BamHI and EcoRI restriction enzymes were obtained from New England BioLabs, USA. SYBR*-Green PCR master mix, 96 well MicroAmp® fast optical reaction plates (0.1 mL capacity) and MicroAmp® optical adhesive films for real-time PCR assay were obtained from Applied Biosystems, Fostercity, CA. All the experiments were performed on StepOnePlus*-Real Time PCR Systems by Applied Biosystems, Fostercity, CA. For amplification of human torque teno virus (TTV), a set of primer pairs described previously were used (Table 3). Primers were made according to the reference strain of TTV genome TA 278 (Gen Bank acc. No. AB008394) and were synthesized by Microsynth (Switzerland) at a scale of 0.2 μmol. DNA ladders, MgCl_2, dNTP’s and buffers were obtained from Fermentas Life sciences, Germany.

**Samples and DNA extraction**

Blood samples (5 mL) collected in EDTA tubes from 20 healthy adult volunteers and 30 randomly selected adult HSCT recipients were centrifuged at 900 g for 10 minutes to separate plasma which was immediately frozen at -20°C until used for DNA extraction. Two independent DNA extractions were performed for each of the healthy individuals along with one independent DNA extraction for HSCT recipients, each from 200 μl of plasma using QIAamp MinElute Virus Spin kit according to the manufacturer’s recommendations. DNA was eluted in 30 μL of Milli-Q water. All extracted DNA samples were stored at -20°C until the analysis. The study protocol was approved by the institution’s ethics committee and healthy donors and HSCT recipient’s samples were used after obtaining informed consent.

**Construction of plasmids for standards preparation**

A region of 119 bp PCR fragment of TTV genome was amplified using primers TTVf and TTVr (Table 3). Resulting amplicon was purified using QIAquick PCR Purification kit, quantified by spectrophotometer and then cloned into the TA cloning vector. The resulting plasmid was transformed into One Shot TOP10F’ competent cells according to instructions provided by the

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| Table 2 SYBR Green PCR assay for TTV using single series of plasmid standards |
|---|
| Plasmid standards | Day | C_T Mean ± SD (n=3)* | Intra-day precision | Inter-day precision | Intra-day accuracy | Inter-day accuracy |
| 10 × 10^9 | 1 | 4.21 ± 0.02 | 0.50 | 0.45 | 99.6 | 99.6 |
| 10 × 10^9 | 2 | 4.18 ± 0.00 | 0.39 | 0.37 | 99.6 | 99.6 |
| 10 × 10^8 | 1 | 7.59 ± 0.05 | 0.75 | 0.65 | 101.3 | 101.3 |
| 10 × 10^8 | 2 | 7.52 ± 0.05 | 0.78 | 0.76 | 101.2 | 101.2 |
| 10 × 10^7 | 1 | 11.74 ± 0.05 | 0.45 | 0.40 | 100.8 | 100.8 |
| 10 × 10^7 | 2 | 11.56 ± 0.00 | 0.14 | 0.13 | 100.8 | 100.8 |
| 10 × 10^6 | 1 | 15.83 ± 0.04 | 0.28 | 0.25 | 100.4 | 100.4 |
| 10 × 10^6 | 2 | 15.56 ± 0.01 | 0.08 | 0.07 | 100.4 | 100.4 |
| 10 × 10^5 | 1 | 19.75 ± 0.05 | 0.26 | 0.24 | 100.6 | 100.7 |
| 10 × 10^5 | 2 | 19.37 ± 0.00 | 0.10 | 0.09 | 100.8 | 100.8 |
| 10 × 10^4 | 1 | 24.15 ± 0.03 | 0.12 | 0.11 | 98.1 | 98.4 |
| 10 × 10^4 | 2 | 23.64 ± 0.02 | 0.10 | 0.09 | 98.6 | 98.6 |
| 10 × 10^3 | 1 | 28.40 ± 0.03 | 0.12 | 0.11 | 95.3 | 95.8 |
| 10 × 10^3 | 2 | 27.75 ± 0.05 | 0.19 | 0.18 | 96.4 | 96.4 |
| 10 × 10^2 | 1 | 33.21 ± 0.20 | 0.62 | 0.59 | 83.8 | 84.3 |
| 10 × 10^2 | 2 | 32.51 ± 0.06 | 0.21 | 0.20 | 84.8 | 84.8 |
| 10 × 10^1 | 1 | 35.59 ± 0.39 | 1.11 | 1.08 | 102.1 | 99.0 |
| 10 × 10^1 | 2 | 35.25 ± 0.06 | 0.17 | 0.16 | 95.7 | 95.7 |

*Tenfold dilutions run in triplicates.
Twelve, isolated colonies of transformed competent cells from solid luria-bertani medium containing ampicillin (100 μg/mL) were subjected for TTV insert confirmation. Each individual colony was suspended separately into 3 mL of liquid luria-bertani medium containing 100 μg/mL of ampicillin for overnight in a shaking incubator at 37°C with a speed of 225 rpm. Following overnight incubation, plasmids purification was done using QIA prep Spin Miniprep kit according to manufacturer’s instructions. Restriction enzyme digestion with EcoRI for the purified plasmids was done to confirm the presence of cloned TTV insert (119 bp) on 1.5% agarose gel electrophoresis (data not shown). TTV insert (119 bp) cloned into TA vector were sequenced for all the 12 separate clones using M-13 forward and reverse primers and confirmed by aligning with the TTV sequence (Gen Bank acc. no. AB008394). This plasmid with TTV inserts was linearized with BamHI enzyme and then used for preparation of standards in serial 10 fold dilutions from 10×10⁹ copies to 20 copies/μL.

### Absolute quantification of TTV DNA

PCR reaction for absolute quantification of TTV DNA using SYBR Green in a 25 μL reaction is as follows: each reaction contained 12.5 μL SYBR Green PCR master mix, 5 μL of template (serial 10 fold dilutions of the linearized plasmid standards or/ extracted DNA from the plasma samples of healthy blood donors), 1.25 μL (500 nm) of each primer (TTVF-1, TTVF-2, TTVR-1, TTVR-2) and 2.5 μL of Milli-Q water. The cycling conditions included initial activation of AmpliTaq Gold DNA polymerase (present in SYBR Green master mix) for 10 minutes at 95°C. The subsequent PCR conditions consisted of 40 cycles of denaturation at 95°C for 15 seconds and annealing and extension at 60°C for 1 minute per cycle. After real-time data acquisition, the cycle threshold value was calculated by determining the point at which the fluorescence exceeds an arbitrary threshold limit. Standards with known TTV DNA copies were prepared in two independent serial dilutions and were run in the range of 100 copies to 10×10⁹ copies on four non-consecutive days to evaluate biological, inter, intraday variability and accuracy of the assay. In addition, a series of standards from one serial dilution were also run in triplicates on two different days to evaluate the intra-day and inter-day variations. The variability of the assay was evaluated by comparing the C₇ values run on the same day (intra-day) and on different days (inter-day) and was represented as co-efficient.
of variations (CV). Accuracy was calculated by taking the ratio of back calculated copies from the standard curve to the theoretical copy number of the reactions. Real-time PCR assay for test samples (HSCT recipients) and for biological replicates of each healthy individual were performed with the inclusion of TTV plasmid standards and negative controls in each run. In addition to this, precision of the assay was also checked by running known TTV positive DNA (positive controls with exact log copies/mL). The viral genomic copies per mL of plasma was calculated as described by Huang et al. [26] i.e., by multiplying the copies per reaction by a factor of 30 [30 μL extracted DNA/5 μL of template x (1 mL/200 μL plasma)].

Melting curve analysis for specificity
Following amplification, melting curve or dissociation curve analysis was performed to measure the specificity of the PCR product. The temperature program used for the melting curve analysis was 95°C for 15 seconds followed by 60°C for 1 minute and then 95°C for 15 seconds with ramp rate of +0.3°C/second.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
AKT and AP performed the experiments. AKT, AP, PHD, CRSU and MA analyzed and contributed to the interpretation of data. MA and ER designed the research. AKT and MA drafted the article. All the authors revised the manuscript critically. All authors read and approved the final manuscript.

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### Table 3: Oligonucleotide primers used for human torque teno virus (TTV)

| Primers ID | Sequence (5’ to 3’) | Nucleotide position* | Reference |
|------------|---------------------|----------------------|-----------|
| TTVf | TCCGAATGCTGCTGTTTCCGAGCGCCGAGCGGGAGAAGGGAGGAGCTTC | 102-118 | Moen EM et al. [19] |
| TTVr | GCGCTCCGAGGGCCGACGCAACCAGCGAGGAGGAGGAGCTTC | 118-210 |
| TTV-F1 | GTTTTGCTCCGAGCAGCGAGGAGGAGGAGGAGCTTC | 115-131 |
| TTV-F2 | GTTTTGCTCCGAGCAGCGAGGAGGAGGAGGAGCTTC | 115-131 |
| TTV-R1 | CCTGACTCCTGCTGCTGCTGCA | 210-192 |
| TTV-R2 | CCTGACTCCTGCTGCTGCTGCA | 210-192 |

*According to the reference strain of TTV genome TA 278 (Gen Bank acc. No. AB008394).
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References

1. King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (Eds): Virus taxonomy: classification and nomenclature of viruses: Ninth Report of the International Committee on Taxonomy of Viruses. 1st edition. San Diego: Elsevier Academic Press; 2012:329–426.
2. Hino S, Miyata H: Torque teno virus (TTV): current status. Rev Med Virol 2007, 17:45–57.
3. Bendinelli M, Pistello M, Maggi F, Fornai C: Molecular Properties, Biology and Clinical Implications of TT Virus, a Recently Identified Widespread Infectious Agent of Humans. Clin Microbiol Rev 2001, 14:98–113.
4. Touinssi M, Gallian P, Biagini P, Attoui H, Vialettes B, Berland Y, Tamalet C, Ghimenti M, Antonelli G, Bendinelli M, Pistello M, Ceccherini-Nelli L, Petrini M: Torquetenovirus viremia kinetics after autologous stem cell transplantation are predictable and may serve as a surrogate for functional immune reconstitution. J Clin Virol 2010, 47:189–192.
5. Okamoto H, Nishizawa T, Kato N, Iikita M, Ikeda H, Iizuka H, Miyakawa Y, Mayumi M: Molecular cloning and characterization of a novel DNA virus (TTV) associated with posttransfusion hepatitis of unknown etiology. Hepatology 1998, 191–196.
6. Mushahwar IK, Erker JC, Muerhoff AS, Leary TP, Simons JN, Birkenmeyer LG, Chalmers ML, Pilot-Matas Tj, Desai SM: Molecular and biophysical characterization of TT virus: Evidence for a new virus family infecting humans. Proc Natl Acad Sci USA 1999, 96:3177–3182.
7. Miyata H, Tsunoda H, Kasi A, Yamada A, Khan MA, Murakami J, Kamatara T, Shiakai K, Hino S: Identification of a novel GC-rich 113-nucleotide region to complete the circular, single-stranded DNA genome of TT virus, the first human circovirus. J Virol 1999, 73:3582–3586.
8. Okamoto H, Nishizawa T, Ukita M, Takahashi M, Ikeda H, Iizuka H, Miyakawa Y, Mayumi M: The entire nucleotide sequence of a TT virus isolate from the United States (TUS01): Comparison with reported isolates and phylogenetic analysis. Virology 1999, 259:437–448.
9. Okamoto H, Nishizawa T, Takahashi M, Asabe S, Tsuda F, Yoshikawa A: Heterogeneous distribution of TT virus of distinct genotypes in multiple tissues from infected humans. Virology 2001, 288:358–368.
10. Okamoto H, Takahashi M, Nishizawa T, Ukita M, Fukuda M, Tsuda F, Miyakawa Y, Mayumi M: Marked genomic heterogeneity and frequent mixed infection of TT virus demonstrated by PCR with primers from coding and noncoding regions. Virology 1999, 259:428–436.
11. Takahashi K, Hijkata M, Samokhvalov EI, Mishiro S: Full or near full length nucleotide sequences of TT virus variants (types SANBAN and YONBAN) and the TT virus-like mini virus. Intervirology 2000, 43:119–123.
12. Muljono DH, Nishizawa T, Tsuda F, Takahashi M, Okamoto H: Molecular epidemiology of TT virus (TTV) and characterization of two novel TTV genotypes in Indonesia. Arch Virol 2001, 146:1249–1266.
13. Moen EM, Selboda J, Grinde B: Real-time PCR methods for independent quantitation of TTV and TLMV. J Virol Methods 2002, 104:59–67.
14. Focosi D, Maggi F, Albani M, Macera L, Ricci V, Gragnani S, Beo SD: Tyagi et al. Virology Journal 2013, 10:191

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