Journal of Clinical Virology 175 (2024) 105738

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Validation, implementation and quality control of a Torque Teno Virus qPCR in a multinational clinical trial

E.J. Gore [a,*], L. Gard [a], P. Bourgeois [b], D. Kulifaj [b], E. McCulloch [c], P.G. Spezia [d], H.G.M. Niesters [a], F. Maggi [d], G. Bond [e], C. Van Leer-Buter [a], TTVguideTX consortium partners[1]

[a] Department of Microbiology and Infection Prevention, University of Groningen. University Medical Center Groningen, Groningen, The Netherlands
[b] bioMérieux SA, 138, Rue Louis Pasteur, Parc Technologique Delta Sud, Verniolle, France
[c] Quality Control for Molecular Diagnostics (QCMD), Glasgow, United Kingdom
[d] Laboratory of Virology, National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS, 00149 Rome, Italy
[e] Medical University of Vienna, Spitalgasse 23, 1090 Vienna, Austria

ARTICLE INFO

Keywords:
TTV
Torque teno virus
Renal transplantation
TTVguideIT
qPCR
Molecular testing

ABSTRACT

Background: Immunosuppressive medication after organ transplantation is usually dosed through therapeutic drug monitoring. Trough levels of antirejection medication however, do not adequately predict rejection or infections. The TTVguideIT trial is a multinational clinical trial evaluating the safety of Torque Teno Virus (TTV) load assessed by qPCR, as an alternative to trough level tacrolimus dosing.
Methods: Prior to, and during the clinical trial, the inter-and intra-laboratory variability, accuracy, and precision of the TTV R-GENE® assay was evaluated through analysis of internal quality control (IQC), external quality assessment (EQA) and linearity panels performed by the thirteen participating clinical virology laboratories, each using their standard testing platforms.
Results: IQC samples with a target of 4 $\log_{10}$ copies/mL (cp/mL) were tested by the participating laboratories 130 times during the implementation phase and 987 times during the trial phase. They showed excellent accuracy, with an inter-laboratory standard deviation (SD) of 0.17 $\log_{10}$ cp/mL, and an intra-laboratory SD of 0.03 to 0.20 $\log_{10}$ cp/mL during the implementation phase, and an inter-laboratory SD of 0.19 $\log_{10}$ cp/mL, and an intra-laboratory SD 0.07 to 0.18 $\log_{10}$ cp/mL during the trial phase. Three EQA panels and three linearity panels showed similarly small variability during implementation as well as within the trial phase.

* Corresponding author.
E-mail address: e.j.gore@umcg.nl (E.J. Gore).
[1] TTV GUIDE Tx consortium partners (www.ttv-guide.eu/team)

- Georg Meizer-Venturi, EUTEMA, Vienna, Austria.
- Christian Hugo, Universitätsklinikum Carl Gustav Carus an der Technischen Universität Dresden, Dresden, Germany.
- David Rodriguez-Arias, Department of Philosophy I, FiloLab-UGR, University of Granada, Granada, Spain.
- Jacques Demotes, ECRIN, Paris, France.
- Isabel Beneyto Castello, Hospital La Fe, Vaencia, Spain.
- Hannes Neuwirt, Department of Internal Medicine IV, Nephrology and Hypertension, Medical University Innsbruck, Innsbruck, Austria.
- Sophie Callard, Les Hospitaux, Universite de Strasbourg, France.
- Stephan J L Bakker, Division of Nephrology, Department of Internal Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.
- Joris I Rotmans, Department of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands.
- Kathrin Eller, Division of Nephrology, Department of Internal Medicine, Medical University of Graz, Graz, Austria.
- Ondrej Viklicky, Transplant Center, Department of Nephrology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.
- Lionel Rostaing, Department of Nephrology, Hemodialysis, Apheresis and Kidney Transplantation, CHU-Grenoble-Alpes, Grenoble, France.
- Miriam Banas, Department of Nephrology, University Hospital Regensburg, Regensburg, Germany.
- Daniel Cejka, Ordensklinikum Linz GmbH Elisabethinen, Linz, Austria.
- Klemens Budde, Charité - Universitätsmedizin Berlin, Berlin, Germany.
- David Navarro, Center for Virology, INCLIVIA, Valencia Spain.
- Paolo Malvezzi, Department of Nephrology, Hemodialysis, Apheresis and Kidney Transplantation, CHU-Grenoble-Alpes, Grenoble, France.

https://doi.org/10.1016/j.jcv.2024.105738
Received 6 August 2024; Received in revised form 3 October 2024;
Available online 16 October 2024

Conclusion: This data shows that TTV load measurement can be standardized for use in a multinational clinical trial. By using IQC, LP and EQA samples, the quality and integrity of the assay can be compared between laboratories and precise and accurate results can be generated.

1. Introduction

Following renal failure, kidney transplantation is the gold standard of treatment with almost 3000 kidney transplants performed in 2022 within the Eurotransplant zone [1]. Following transplantation, patients must take immunosuppressive medication (IS) for life. Currently, IS is dosed either through a protocol-based regime or through therapeutic drug monitoring (TDM) schemes [2,3]. IS levels must stay within a predetermined range of trough levels to reduce the risk of rejection on the one hand and malignancy or infection on the other hand [4,5]. An important limitation of dosing via TDM is that trough levels of immunosuppressants are poor predictors of such events and only reflect the previous 24 hours of drug therapy [6,7].

Recently, Torque Teno virus (TTV) has been proposed to guide IS dosing. TTV is a small, circular, single-stranded DNA virus classified into the family *Anelloviridae* and characterized by a high prevalence. >20 species of TTV have been identified in humans, each of which is categorized by variable numbers of genotypes [8–10]. TTV does not cause pathology but can be detected and quantified in the plasma, whole blood, or serum of immunocompromised and healthy individuals. It has been suggested that TTV load in the blood is low when patients are insufficiently immunosuppressed and at risk of rejection [11–17] and high in over-immunosuppressed patients at risk for infections [12,13,15, 18–20]. A major drawback of previous research is that in-house PCR-based protocols have been utilized, potentially with differing sensitivity to individual genotypes, resulting in calls for assay standardization for use in clinical studies [5,21].

The randomized controlled TTVguideIT trial was designed to investigate if TTV load can be used to adjust the immunosuppressive drug tacrolimus in renal transplant recipients (RTR) to reduce graft rejection and infection. Thirteen clinical centers in six European countries are participating in TTVguideIT, and are determining the TTV load locally using the TTV R-GENE® IVDR assay (bioMérieux, Lyon, France). Based on previous data, the TTV load targeted during the trial was determined to be between 4.6 and 6.2 $\log_{10}$ cp/mL [22,23]. Tacrolimus dose adjustments are made when participants have measurements outside this range. This implies that the accuracy and reproducibility of the TTV assay are of vital importance and had to be established in each center before patients were recruited.

In this study, we report on the implementation and quality assessment of the assay prior to and during the TTVguideIT trial. Inter- and intra-laboratory variability and the accuracy and precision of the assay are evaluated.

2. Methods and materials

Fifteen laboratories participated in the inter-laboratory comparison of the TTV R-GENE® assay, of which thirteen participated in the clinical TTVguideIT trial with two expert centers, each using their standard DNA extraction and amplification platforms. Participating laboratories and platforms used in the TTVguideIT trial are detailed in Table 1. The TTV R-GENE® assay was used by all participants to detect and quantify TTV during implementation, and the active phase of the trial. Results are expressed in $\log_{10}$ cp/mL, with a limit of detection of 2.4 $\log_{10}$ cp/mL [24]. Validation of individual platforms is outlined in the supplementary data (supplementary figure 1A - 1D and supplementary figure 2A to 2D).

2.1. Quality assurance materials

A stock solution for use as an Internal Quality Control (IQC) containing only TTV species 22 was diluted using negative donor plasma to a standardized sample containing 4 $\log_{10}$ cp/mL, as determined by digital-droplet PCR (QCMD, Glasgow, UK/Spallanzani National Institute for Infectious Diseases, Rome, Italy). A total of 1400 samples manufactured to ISO-13485 were generated for use in the implementation and experimental phases of the trial in two batches. According to the test protocol, the IQC data must conform to the rules laid out in Table 2, which also details the actions to be taken if the IQC is outside the preset range.

Linearity was determined by all participating laboratories during the implementation and trial phases by using a linearity panel (LP) of six samples with a dynamic range from 4 to 8 $\log_{10}$ cp/mL. It was prepared by diluting a TTV stock of species 8 plasmids in transport medium (Spallanzani National Institute for Infectious Diseases, Rome, Italy) and verified by sequencing and digital droplet PCR.

An external quality assessment (EQA) panel (QCMD, Glasgow, UK) was designed in line with ISO-17043 proficiency testing requirements using a combination of patient samples and materials made with plasmids to ensure that a variety of TTV concentrations and TTV species were detected. Samples were blinded to the participating laboratories and results returned to QCMD via a dedicated online reporting system.

Table 1
Extraction and amplification methods used in each centre.

Extraction and amplification method	Centre
EMAG and CFX96	Laboratory 10
	Laboratory 9*
	Laboratory 11
EMAG and ABI 7500	Laboratory 12
EMAG and LightCycler 480 (System II)	Laboratory 3
	Laboratory 4
	Laboratory 8
EMAG Rotor-Gene Q	Laboratory 6
EasyMAG and LightCycler 480 (System II)	Laboratory 2
	Laboratory 10
EasyMAG and CFX96	Laboratory 9*
MagNA Pure 96 and CFX96	Laboratory 7
MagNA Pure 96 and QuantStudio 5	Laboratory 13
QIAsymphony SP and QuantStudio 5	Laboratory 5

* EMAG from September 2023

EMAG/easyMAG (bioMérieux, France). MagNA Pure 96 (Roche, Germany). QIAsymphony SP (QIAGEN, Germany). CFX96 (Bio-Rad, USA). Rotor-Gene Q (QIAGEN, Germany). LightCycler 480 (System II) (Roche, Germany). ABI 7500 (Thermo Fisher Scientific, USA).

Table 2
IQC rejection criteria and action points.

Rule	Description	Comments
Rule 1	1 point >3 standard deviations from the mean.	Result Not Conform: investigate the drift & retest
Rule 2	2 of 3 consecutive points greater than 2 standard deviations from the mean on the same side.	Result Not Conform: investigate the drift & retest
Rule 3	4 consecutive points greater than 1 standard deviation from the mean on the same side.	Result Conform: investigate the drift
Rule 4	7 consecutive points progressing higher or lower in the same direction.	Result Conform: investigate the drift
Rule 5	9 consecutive points in a row on the same side of the mean.	Result Conform: investigate the drift

Table 3
Timeframe of when EQA and linearity panels were issued.

	Period	Number of laboratories
Validation phase		
EQA panel 1	Second quarter 2022	13
Linearity panel 1	Second quarter 2022	13
Trial phase		
EQA panel 2	Second quarter 2023	13
Linearity panel 2	Second quarter 2023	13
EQA panel 3	Second quarter 2024	13
Linearity panel 3	Second quarter 2024	13

2.2. Quality assurance and assessment during the trial phase

IQC was performed with each run of patient material and submitted to the IQC monitoring interface on the QCMD website. EQA panel runs were performed annually in the trial phase along with one linearity panel. The mean and SD of each EQA sample were determined by calculating the mean and SD of the values of all participating laboratories. Table 3 outlines when each EQA assessment took place.

3. Results

3.1. IQC during implementation

All 130 results obtained during the implementation phase were valid giving a mean of 3.96 $\log_{10}$ cp/mL with a target of 4 $\log_{10}$ cp/mL, range: 3.44 to 4.28 $\log_{10}$ cp/mL; Fig. 1A. A total of 78 % of results were within 1 SD of the target, giving an inter-laboratory SD of 0.17 $\log_{10}$ cp/mL, and an intra-laboratory SD between 0.03 and 0.20 $\log_{10}$ cp/mL. Laboratories 5, 6, 8, 9, and 13 showed a mean below the target, and laboratories 3, 11, and 12 were above the target. Laboratories 1, 2, 4, 7, and 10 were within 0.05 $\log_{10}$ cp/mL. Laboratory 13 had the lowest accuracy, with a mean of 3.61 $\log_{10}$ cp/mL.

3.2. Results of IQC after the start of the trial

During the trial phase of 1014 TTV-detection runs, 27 were rejected (2.7 %) due to IQC criteria detailed in Table 2 not being fulfilled. Data with the non-rejected runs are included as supplementary information (supplementary figure 3A). In four cases, the reported IQC value was >3 SD from the required mean, and in the remaining 23 cases the IQC value was outside the 2 SD margin from the mean in two out of three sequential samples. The analysis of the remaining 987 IQC results showed a mean of 4.0 $\log_{10}$ cp/mL (target 4.0 $\log_{10}$ cp/mL, range: 3.43 to 4.58 $\log_{10}$ cp/mL; Fig. 1B), an inter-laboratory SD of 0.19 $\log_{10}$ cp/mL and an intra-laboratory SD between 0.07 and 0.18 $\log_{10}$ cp/mL. This corresponded to an average of 76 results per laboratory, with a range of 31–190. Laboratories 1, 4, 5, 8, 9, and 13 were below the target, laboratories 11 and 12 were above the target, and laboratories 2, 3, 6, 7, and 10 were within 0.05 $\log_{10}$ cp/mL. Laboratory 13 had the lowest accuracy with a mean of 3.70 $\log_{10}$ cp/mL.

3.3. EQA and linearity panel testing

All 13 laboratories submitted data for all linearity panels and the EQA testing program during both the implementation and trial phases. EQA Panel 1 had an SD range from 0.13 to 0.25 log10 cp/mL for the seven positive dilution samples (Fig. 2A). Linearity panel 1 (LP1) had an SD range from 0.10 to 0.17 $\log_{10}$ cp/mL for the five positive dilution samples and an R^2 between 0.99 and 1.00 (Fig. 2B). Lower than expected results were seen in samples five and six of EQA Panel 1 (Fig. 2A) and in samples three and four of LP1 (Fig. 2B) in tests conducted by laboratory 13, which uses the MagNA Pure extraction system (Roche Diagnostics, Germany).

EQA Panel 2 had an SD range from 0.14 to 0.23 $\log_{10}$ cp/mL for the seven positive samples, all sites correctly identified the negative sample (Fig. 3A). Linearity panel 2 (LP2) had an SD range from 0.14 to 0.21 $\log_{10}$ cp/mL for the five positive dilution samples and an R^2 between 0.99 and 1.00 (Fig. 3B).

EQA Panel 3 had an SD range from 0.17 to 0.26 $\log_{10}$ cp/mL for the seven positive samples, all sites correctly identified the negative sample (Fig. 3C). Linearity panel 3 (LP3) had an SD range from 0.16 to 0.22 $\log_{10}$ cp/mL for the five positive dilution samples and an R^2 between 0.99 and 1.00 (Fig. 3D).

3.4. Platform performance

Of the thirteen participating clinical centers seven used a unique combination of extraction and amplification platforms (Table 1, Fig. 4A). However, laboratories 1, 9, and 11, used the EMAG/CFX96 combination, giving a mean of 4.03 (range: 3.66 to 4.39) and an SD of 0.20 $\log_{10}$ cp/mL. Further, 55 % of the results were within 1 SD of the target value and 100 % within 2 SD. Laboratories 3, 4, and 8 used the EMAG/Lightcycler 480 combination giving a mean of 3.91 (range: 3.51 to 4.48 $\log_{10}$ cp/mL) and an SD of 0.16 $\log_{10}$ cp/mL. A total of 70 % of results with this combination were within 1 SD and 97 % were within 2 SD of the target. These results suggest that the EMAG/CFX96 combination is more accurate than the EMAG/Lightcycler 480 combination as indicated by a closer-to-target average but less precise as indicated by the larger SD of measurements.

3.5. New batch IQC

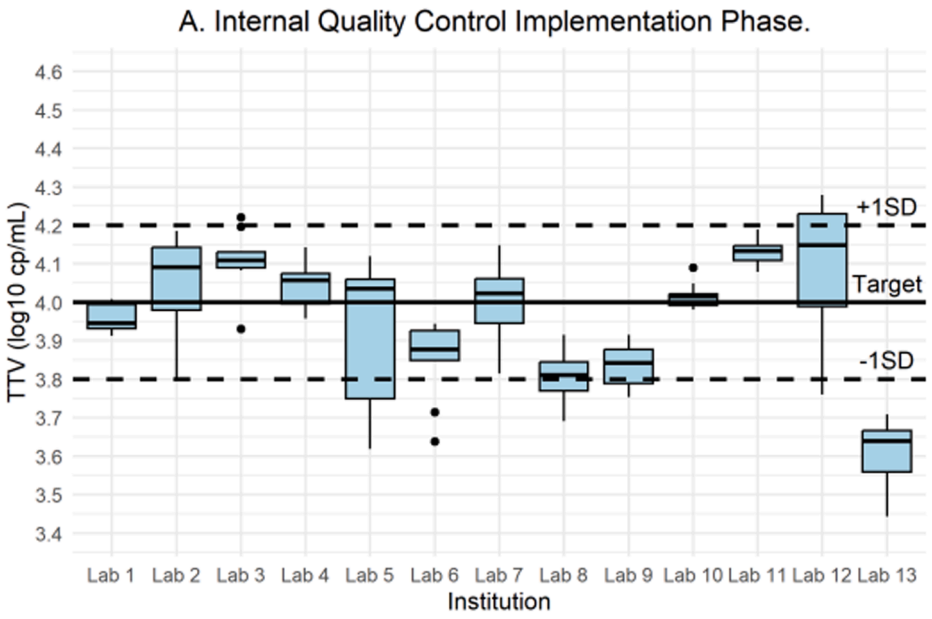
There was no difference between the two IQC batches used during the implementation and trial phases (Fig. 4B). Batch one was run 637 times with a mean of 4.03 $\log_{10}$ cp/mL and an SD of 0.18 $\log_{10}$ cp/mL. Batch two was run 350 times, with a mean of 3.95 $\log_{10}$ cp/mL and an SD of 0.20 $\log_{10}$ cp/mL.

4. Discussion

Within this study, we analyzed the implementation and post-implementation IQC and EQA assessments of the R-GENE® TTV qPCR centers in the context of a TTV-load-based interventional trial in kidney transplant recipients. As part of the trial, dose adjustments of antirejection drugs are made when TTV-loads are outside the predetermined optimal range, necessitating great accuracy of TTV-measurements by all participating centers, who used their own platforms to run the test. Despite nine different platform combinations, thirteen clinical centers, being situated in six different countries it was possible to generate test results with excellent accuracy, precision and reproducibility. This is shown by the small inter-laboratory and intra-laboratory SD of 0.19 $\log_{10}$ cp/mL and 0.07 and 0.18 $\log_{10}$ cp/mL respectively. The accuracy of the test in the entire range of quantitative measurements was verified in the linearity and EQA panels, results of which had SD ranges between 0.10 and 0.26.

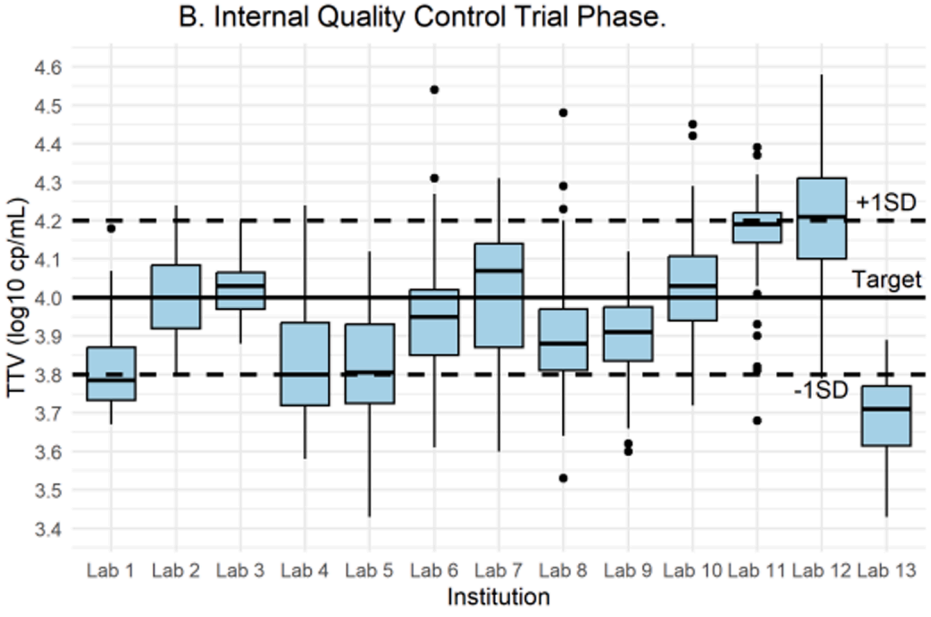
Some minor differences can nevertheless be observed, which may be attributable to the different platform combinations used by the participants. As three laboratories used the EMAG/CFX96 combination and three used the EMAG/Lightcycler 480 combination, we found that the EMAG/CFX96 combination was marginally but not significantly more accurate with a mean closer the target than the EMAG/Lightcycler 480 combination, but less precise with a greater SD.

Lower than expected results were seen in samples three and four of LP1 (Fig. 2B) and samples five and six of EQA Panel 1 (Fig. 2A) in runs conducted by laboratory 13, which uses the MagNA Pure extraction system (Roche Diagnostics, Germany). These non-conform results are theorized to have been caused by the MagNA Pure extraction system, which is less efficient at extracting DNA from transport medium. This problem was previously observed at this site using the MagNA Pure on samples diluted in transport medium for other runs (data not shown). No subsequent issues were observed with this site in either LP or EQA panels.



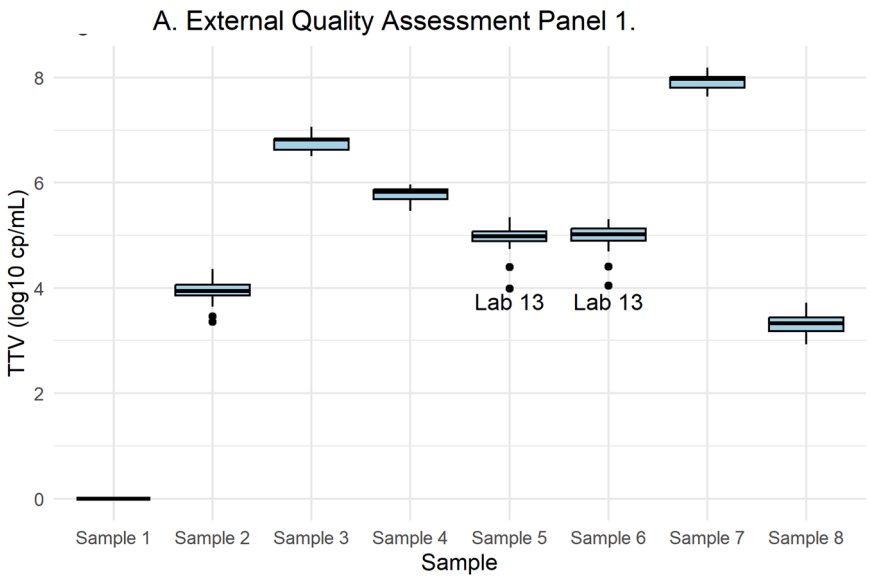
Laboratory	1	2	3	4	5	6	7	8	9	10	11	12	13
Mean	3.96	4.05	4.11	4.04	3.92	3.85	4.00	3.80	3.84	4.02	4.13	4.09	3.61
Min	3.91	3.80	3.93	3.96	3.62	3.64	3.81	3.69	3.75	3.98	4.08	3.76	3.44
Max	4.01	4.18	4.22	4.14	4.12	3.94	4.15	3.91	3.92	4.09	4.19	4.28	3.71
SD	0.04	0.12	0.08	0.06	0.20	0.10	0.10	0.07	0.06	0.03	0.03	0.17	0.08

10 samples per laboratory



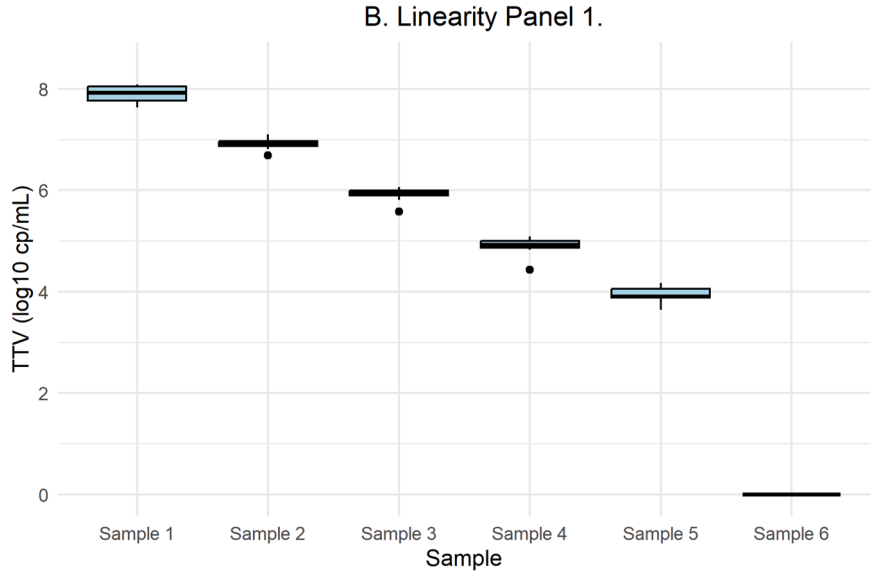
Laboratory	1	2	3	4	5	6	7	8	9	10	11	12	13
N	58	31	39	43	40	60	69	65	99	190	98	161	34
Mean	3.81	4.00	4.02	3.83	3.82	3.95	4.01	3.90	3.90	4.03	4.17	4.20	3.70
Min	3.67	3.80	3.88	3.58	3.43	3.61	3.60	3.33	3.60	3.72	3.68	3.79	3.43
Max	4.18	4.24	4.20	4.24	4.12	4.54	4.31	4.48	4.12	4.45	4.39	4.58	3.89
SD	0.11	0.12	0.08	0.14	0.16	0.16	0.18	0.18	0.10	0.12	0.11	0.16	0.12

Fig. 1. Boxplots showing Internal Quality Control (IQC) data for (A) the implementation phase with 130 runs, mean of 3.96 $\log_{10}$ cp/mL, target 4 $\log_{10}$ cp/mL, range: 3.44 to 4.28 $\log_{10}$ cp/mL, inter-laboratory standard deviation (SD) of 0.17 $\log_{10}$ cp/mL and (B) the trial phase with 987 IQC results a mean of 4.00, range: 3.43 to 4.58 $\log_{10}$ cp/mL, an inter-laboratory SD of 0.19 $\log_{10}$ cp/mL and an intra-laboratory SD between 0.07 and 0.18 $\log_{10}$ cp/mL.



Sample	1	2	3	4	5	6	7	8
N	13	13	13	13	13	13	13	13
Min	0	3.36	6.50	5.62	3.99	4.05	7.64	2.93
Max	0	4.36	6.95	5.96	5.34	5.31	8.18	3.72
Mean	0	3.94	6.77	5.80	4.93	4.96	7.92	3.31
SD	0	0.25	0.13	0.14	0.32	0.32	0.15	0.23

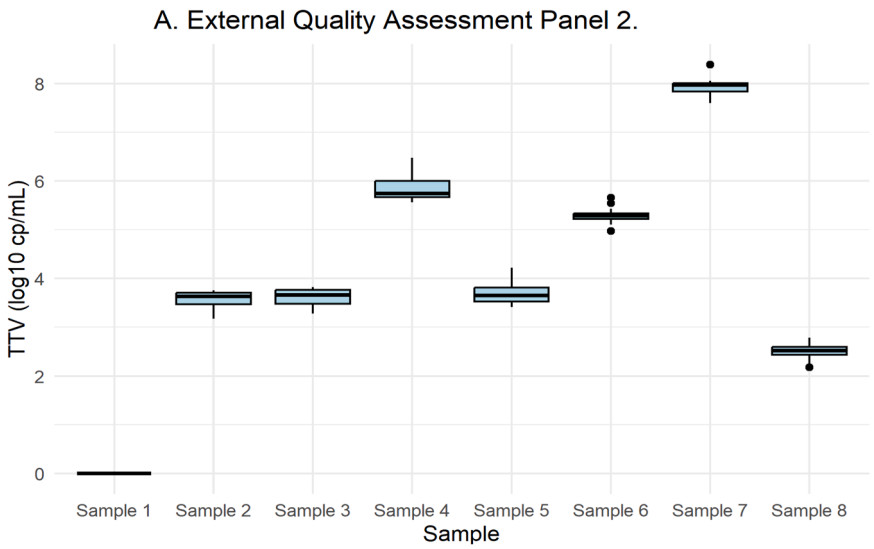
(log10 cp/mL)



Sample	1	2	3	4	5	6
N	13	13	13	13	13	13
Min	7.64	6.69	5.58	4.43	3.64	0
Max	8.08	7.10	6.07	5.09	4.17	0
Mean	7.90	6.91	5.92	4.92	3.95	0
SD	0.16	0.10	0.12	0.17	0.16	0

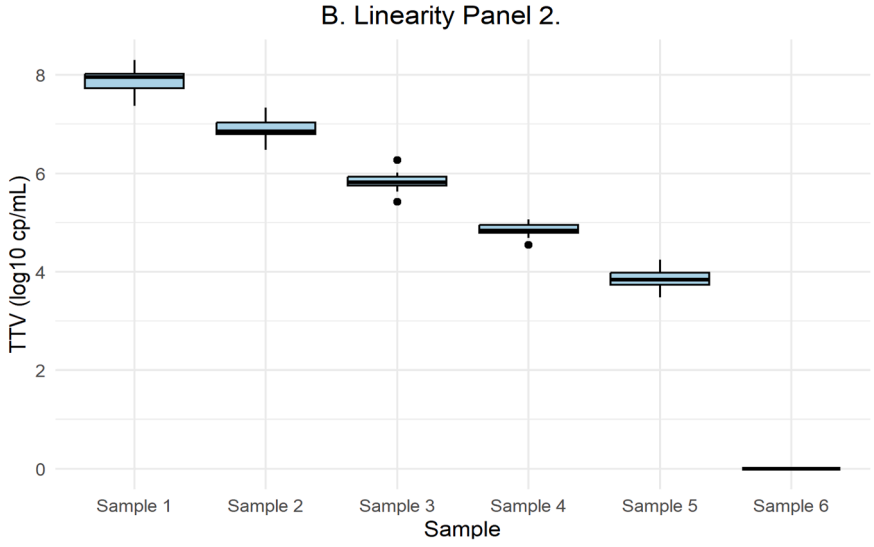
(log10 cp/mL)

Fig. 2. External quality assessment (EQA) and linearity panels from the implementation of the assay. All 13 laboratories correctly submitted data for both for EQA Panel 1 (A) and linearity panel 1 (LP1) (B). EQA Panel 1 had an a standard deviation (SD) range from 0.13 to 0.25 for the seven positive dilution samples. LP1 had an SD range from 0.10 to 0.17 $\log_{10}$ cp/mL for the five positive dilution samples, and an R^2 between 0.99 and 1.00.



Sample	1	2	3	4	5	6	7	8
N	13	13	13	13	13	13	13	13
Min	0	3.17	3.28	5.57	3.42	4.98	7.60	2.17
Max	0	3.76	3.82	6.61	4.22	5.54	8.06	2.17
Mean	0	3.57	3.61	5.83	3.70	5.27	7.90	2.52
SD	0	0.18	0.18	0.23	0.23	0.14	0.14	0.17

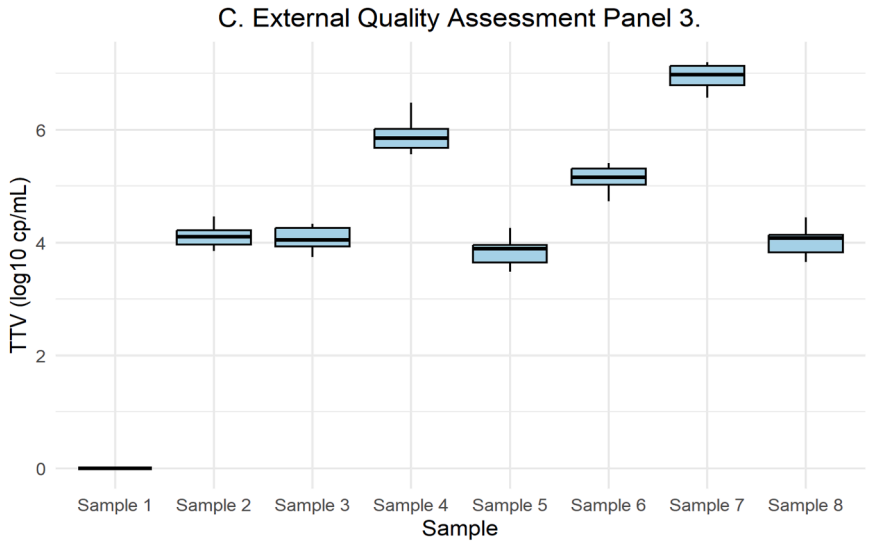
(log10 cp/mL)



Sample	1	2	3	4	5	6
N	13	13	13	13	13	13
Min	7.37	6.47	5.42	4.55	3.48	0
Max	8.08	7.27	6.01	5.06	4.05	0
Mean	7.87	6.87	5.81	4.85	3.84	0
SD	0.20	0.21	0.16	0.14	0.16	0

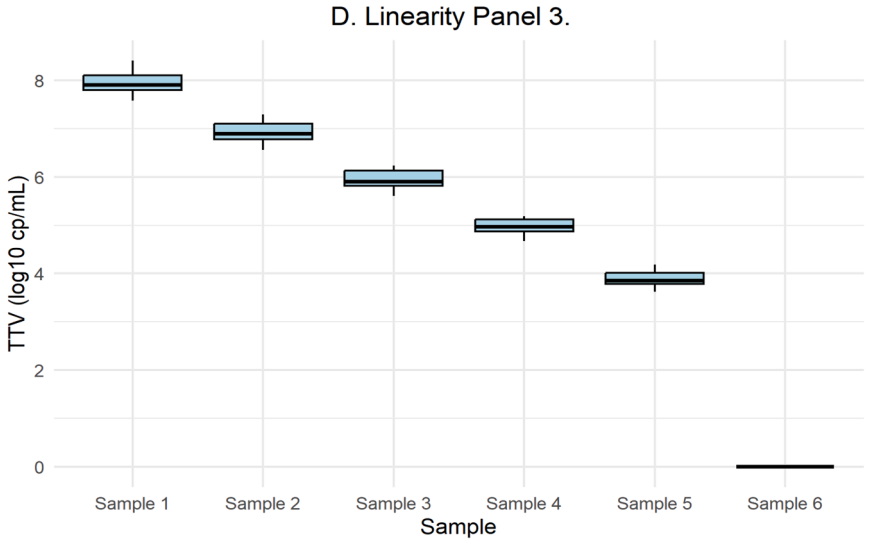
(log10 cp/mL)

Fig. 3. All 13 laboratories submitted data for the external quality assessment (EQA) and linearity panels from the trial phase. (A) EQA Panel 2 had an standard deviation (SD) range from 0.14 to 0.23 $\log_{10}$ cp/mL for the seven positive samples, all sites correctly identified the negative sample. (B) Linearity panel 2 had an SD range from 0.14 to 0.21 $\log_{10}$ cp/mL for the five positive dilution samples, and an R^2 between 0.99 and 1.00. (C) EQA Panel 3 had an SD range from 0.17 to 0.26 $\log_{10}$ cp/mL for the seven positive samples, all sites correctly identified the negative sample. (D) Linearity panel 3 had an SD range from 0.16 to 0.22 $\log_{10}$ cp/mL for the five positive dilution samples and an R^2 between 0.99 and 1.00.



Sample	1	2	3	4	5	6	7	8
N	13	13	13	13	13	13	13	13
Min	0	3.85	3.75	5.57	3.52	4.73	6.57	3.66
Max	0	4.46	4.33	6.48	4.26	5.41	7.20	4.45
Mean	0	4.11	4.09	5.92	3.89	5.14	6.94	4.01
SD	0	0.17	0.20	0.29	0.24	0.21	0.21	0.26

(log10 cp/mL)

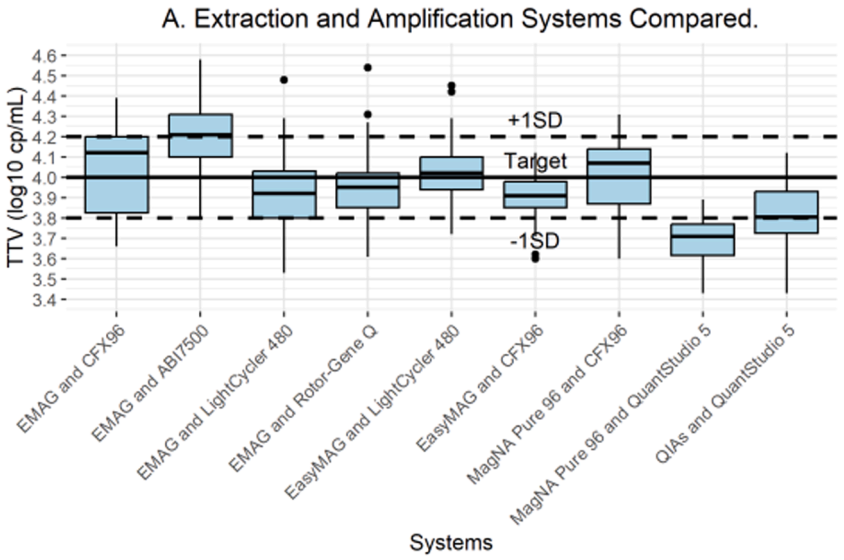


Sample	1	2	3	4	5	6
N	13	13	13	13	13	13
Min	7.58	6.56	5.60	4.69	3.63	0
Max	8.41	7.29	6.23	5.18	4.19	0
Mean	7.96	6.94	5.94	4.99	3.89	0
SD	0.21	0.22	0.19	0.16	0.18	0

(log10 cp/mL)

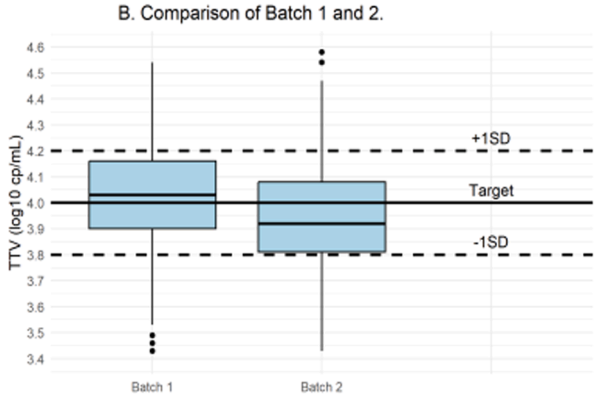
Fig. 3. (*continued*).

Repeat IQC testing was used to assess the reproducibility, accuracy and precision during all phases of the trial. The IQC has the predetermined concentration of 4.0 $\log_{10}$ cp/mL, and rules were set as to how far from this value results can be before the run is rejected and repeated (Table 2). This study shows that each laboratory generated results with high reproducibility, and that intra-laboratory variation was smaller than the inter-laboratory variation. Laboratories measuring higher loads than the predetermined 4.0 $\log_{10}$ cp/mL, did so consistently, as did laboratories measuring below 4.0 $\log_{10}$ cp/mL. In the TTVguideIT trial, tacrolimus dosing adjustments are made if the TTV load is outside the target range of 4.6 $\log_{10}$ to 6.2 $\log_{10}$ cp/mL [22,23]. Therefore, a patient enrolled in a center, e.g., with an IQC mean which is



	EMAG/ CFX96	EMAG/ ABI7500	EMAG/ LightCycler	EMAG/ RotorGene	EasyMAG/ LightCycler	EasyMAG/ CFX96	MagNA/ CFX96	MagNA/ QS	QIAS/ QS
N	162	165	147	60	221	93	69	34	40
Mean	4.03	4.20	3.91	3.95	4.03	3.91	4.01	3.70	3.82
Min	3.66	3.79	3.53	3.61	3.72	3.60	3.60	3.43	3.43
Max	4.39	4.58	4.48	4.54	4.45	4.12	4.31	3.89	4.12
SD	0.20	0.16	0.16	0.16	0.12	0.10	0.18	0.12	0.16

QS: Quantstudio . (log10 cp/mL)



	Batch 1	Batch 2
N	637	350
Mean	4.03	3.95
Min	3.43	3.43
Max	4.54	4.58
SD	0.18	0.20

(log10 cp/mL)

Fig. 4. Comparison of extraction and amplification system combinations (A) laboratories 1, 9 and 11, used the EMAG®/CFX96 combination giving a mean 4.03, range: 3.66 to 4.39, and an standard deviation (SD) of 0.2 $\log_{10}$ cp/mL. Laboratories 3, 4 and 8 used the EMAG®/Lightcycler 480 combination giving a mean 3.91, range: 3.51 to 4.48, and an SD of 0.16 $\log_{10}$ cp/mL. (B) There was no difference between two IQC batches used during validation and trial phases. Batch one was run 637 times with a mean of 4.03 $\log_{10}$ cp/mL and an SD of 0.18 $\log_{10}$ cp/mL. Batch two was run 350 times, with a mean of 3.95 $\log_{10}$ cp/mL and an SD of 0.20 $\log_{10}$ cp/mL.

lower than the inter-laboratory mean may be more likely to have dose adjustments upward, potentially leading to a net increase in tacrolimus dosing. Given the structural bias exhibited by certain laboratories, possibly caused by platform variation, we suggest that it would be beneficial to use the 4.0 $\log_{10}$ cp/mL sample as a calibrator, to apply a correction based on the IQC variation individually calculated for each laboratory using the mean ± the SD for each laboratory. Analysis of the frequency and quantity of tacrolimus dosage adjustments that occurred in each individual center will give more information regarding the potential impact caused by variation between sites. This approach could be tested in upcoming trials such as TAOIST [25].

Limitations of this study include that there is a large variation in the number of IQC runs performed between centers, with some centers performing four times the IQC testing compared to others. This could be reflected in the differences in SD variation between the IQC runs in the implementation and trial phase (Fig. 1A, and B). Also, we cannot be certain that differences between laboratories are the result of extraction and amplification platforms. To investigate the impact of each platform, a much larger number of laboratories will have to be included.

This study shows that it is possible to standardize a TTV qPCR across multiple centers throughout Europe and obtain accurate and repeatable data. In the past few years, there have been calls for TTV qPCR standardization by many authors, to allow for the direct comparison of TTV loads across different centers [5,21]. Such standardization has been achieved in this study using a single qPCR test. However, another way of standardizing laboratory reporting is through EQA testing, and by using an international standard, such as the IQC material used it this validation. EQA panels are, at time of writing, available through QCMD. The TTV R-GENE assay currently includes a run control which is comparable to the IQC utilized in this study. As TTV gains more attraction as a biomarker in transplantation and other fields such as rheumatology, oncology and infectious disease, an international standard, such as those provided through the World Health Organization for other blood-borne viruses[26], should be made available with high priority.

CRediT authorship contribution statement

E.J. Gore: Writing – review & editing, Writing – original draft, Formal analysis. **L. Gard:** Writing – review & editing, Methodology. **P. Bourgeois:** Writing – review & editing, Methodology. **D. Kulifaj:** Writing – review & editing, Formal analysis, Data curation. **E. McCulloch:** Writing – review & editing, Methodology, Investigation. **P.G. Spezia:** Writing – review & editing, Methodology. **H.G.M. Niesters:** Writing – review & editing, Supervision. **F. Maggi:** Writing – review & editing. **G. Bond:** Writing – review & editing, Supervision, Conceptualization. **C. Van Leer-Buter:** Writing – review & editing, Writing – original draft, Supervision.

Declaration of competing interest

P. Bourgeois and D. Kulifaj are current employees of bioMérieux. G. Bond previously received a payment from bioMérieux for the preparation of an information brochure on TTV R-GENE for an invited talk on TTV at a scientific conference. No other authors have any conflicts of interest to declare.

Funding

This work is published on behalf of the consortium of the TTVguideTX project, funded by the European Union's Horizon 2020 research and innovation program under grant agreement number 896932.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2024.105738.

References

[1] Eurotransplant. Eurotransplant Annual Report 2022. 2022.
[2] J. Bamoulid, O. Staeck, F. Halleck, D. Khadzhynov, S. Brakemeier, M. Dürr, et al., The need for minimization strategies: current problems of immunosuppression, Transplant Int. 28 (8) (2015) 891–900. Aug.
[3] Jonge H De, Naesens M, D.R.J Kuypers, New insights into the pharmacokinetics and pharmacodynamics of the calcineurin inhibitors and mycophenolic acid: possible consequences for therapeutic drug monitoring in solid organ transplantation, Ther. Drug Monit. 31 (4) (2009) 416–435.
[4] A. van Rijn, R. Roos, F. Dekker, J. Rotmans, M. Feltkamp, Torque teno virus load as marker of rejection and infection in solid organ transplantation – a systematic review and meta-analysis, Rev. Med. Virol. (2023). Jan 3.
[5] E.J. Gore, L. Gard, H.G.M. Niesters, C.C Van Leer Buter, Understanding torquetenovirus (TTV) as an immune marker, Front. Med. (Lausanne) 10 (2023). June.
[6] R. Bouamar, N. Shuker, D.A. Hesselink, W. Weimar, H. Ekberg, B. Kaplan, et al., Tacrolimus predose concentrations do not predict the risk of acute rejection after renal transplantation: a pooled analysis from three randomized-controlled clinical trials, Am. J. Transplant. 13 (5) (2013) 1253–1261. May.
[7] C. Percy, Z. Hassoun, M. Mourad, M. De Meyer, C. Beguin, M. Jadoul, et al., Impact of acute infection requiring hospitalization on tacrolimus blood levels in kidney transplant recipients, TransPlant. Proc. 49 (9) (2017) 2065–2069. Nov.
[8] S. Spandole, D. Cimponeriu, L.M. Berca, G. Mihaescu, Human anelloviruses: an update of molecular, epidemiological and clinical aspects, Arch. Virol. 160 (4) (2015) 893–908. Apr.
[9] A. Varsani, T. Opriessnig, V. Celer, F. Maggi, H. Okamoto, A.L. Blomström, et al., Taxonomic update for mammalian anelloviruses (family Anelloviridae), Arch. Virol. 166 (10) (2021) 2943–2953. Oct 1.
[10] N. Redondo, D. Navarro, J.M. Aguado, M. Fernández-Ruiz, Viruses, friends, and foes: the case of Torque Teno Virus and the net state of immunosuppression, Transplant Infect. Dis. 24 (2) (2022). Apr 31.
[11] M. Schiemann, E. Puchhammer-Stöckl, F. Eskandary, P. Kohlbeck, S. Rasoul-Rockenschaub, A. Heilos, et al., Torque Teno Virus load-inverse association with antibody-mediated rejection after kidney transplantation, Transplantation 101 (2) (2017) 360–367. Feb.
[12] M. Solis, A. Velay, P. Gantner, J. Bausson, A. Filiputti, R. Freitag, et al., Torquetenovirus viremia for early prediction of graft rejection after kidney transplantation, J. Infect. (2019).
[13] M. Fernández-Ruiz, E. Albert, E. Giménez, T. Ruiz-Merlo, P. Parra, F. López-Medrano, et al., Monitoring of alphatorquevirus DNA levels for the prediction of immunosuppression-related complications after kidney transplantation, Am. J. Transplant. 19 (4) (2019) 1139–1149. Apr 10.
[14] R. Strassl, K. Doberer, S. Rasoul-Rockenschaub, H. Herkner, I. Görzer, J.P. Kläger, et al., Torque teno virus for risk stratification of acute biopsyproven alloreactivity in kidney transplant recipients, J. Infect. Dis. 219 (12) (2019) 1934–1939. Jan.
[15] K. Doberer, M. Schiemann, R. Strassl, F. Haupenthal, F. Dermuth, I. Görzer, et al., Torque teno virus for risk stratification of graft rejection and infection in kidney transplant recipients—a prospective observational trial, Am. J. Transplant. 20 (8) (2020) 2081–2090. Aug 8.
[16] K. Masutani, Viral infections directly involved in kidney allograft function, Nephrology 23 (S2) (2018) 31–37. Jul 2.
[17] K. Doberer, F. Haupenthal, M. Nackenhorst, F. Bauernfeind, F. Dermuth, M. Eigenschink, et al., Torque Teno Virus load is associated with subclinical alloreactivity in kidney transplant recipients: a prospective observational trial, Transplantation 105 (9) (2021) 2112–2118. Sep 19.
[18] F. Maggi, D. Focosi, M. Statzu, G. Bianco, C. Costa, L. Macera, et al., Early post-transplant torquetenovirus viremia predicts cytomegalovirus reactivations in solid organ transplant recipients, Sci Rep. 8 (1) (2018) 15490–15498.
[19] R. Strassl, M. Schiemann, K. Doberer, I. Görzer, E. Puchhammer-Stöckl, F. Eskandary, et al., Quantification of Torque Teno Virus Viremia as a prospective biomarker for infectious disease in kidney allograft recipients, J. Infect. Dis. 218 (8) (2018) 1191–1199. Sep.
[20] M. Fernández-Ruiz, E. Albert, E. Giménez, I. Rodríguez-Goncer, A. Andrés, D. Navarro, et al., Early kinetics of Torque Teno virus DNA load and BK polyomavirus viremia after kidney transplantation, Transplant Infect. Dis. 22 (2) (2020). Apr 9.
[21] D. Focosi, F. Maggi, Torque teno virus monitoring in transplantation: the quest for standardization, Am. J. Transplant. 19 (5) (2019) 1599–1601. May 17.
[22] I. Görzer, F. Haupenthal, F. Maggi, F. Gelas, D. Kulifaj, L. Brossault, et al., Validation of plasma Torque Teno viral load applying a CE-certified PCR for risk stratification of rejection and infection post kidney transplantation, J. Clin. Virol. (2023) 158. Jan 1.
[23] F. Haupenthal, J. Rahn, F. Maggi, F. Gelas, P. Bourgeois, C. Hugo, et al., A multicentre, patient- and assessor-blinded, non-inferiority, randomised and controlled phase II trial to compare standard and torque teno virus-guided immunosuppression in kidney transplant recipients in the first year after transplantation: tTVguideIT, Trials 24 (1) (2023) 213. Mar 22.
[24] bioMérieux. TTV R-GENE Real-time detection and quantification assay. Instructions for Use. Package Insert - 423414. 2022.
[25] S. Kapps, F. Haupenthal, G. Bond, Torque Teno Virus–guided monitoring of immunosuppressive therapy, Nephrol. Dial. Transplant. (2024). Jun 26.
[26] S.A. Baylis, P. Wallace, E. McCulloch, H.G.M. Niesters, C.M. Nübling, Standardization of nucleic acid tests: the approach of the World Health Organization, J. Clin. Microbiol. 57 (1) (2019). Jan.