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# Comparison of FTD SARS-CoV-2 Assay and RealStar RT-PCR kit 1.0 for the detection of SARS-CoV-2

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## Highlights

- Evaluation of COVID-19 diagnostic assays is crucial for the choice of an appropriate molecular test.
- To date, there are no comparison data for FTD SARS-CoV-2 Assay.
- Comparison of FTD SARS-CoV-2 Assay and RealStar RT-PCR kit 1.0 showed a substantial agreement.
- Discrepancies were found for results associated with higher cycle threshold values.

## Abstract

The aim of the study was to evaluate the clinical performance of FTD SARS-CoV-2 compared to the RealStar RT-PCR kit 1.0. The analysis of 100 nasopharyngeal swabs showed an overall agreement of 88%. The positive percentage agreement was 85.6% and the negative percentage agreement was 91%. In conclusion we observed a substantial agreement among the two methods, with discrepancies mainly observed in specimens with relatively low amount of viral RNA.

**Key word (3-6):** Covid-19, FTD SARS-CoV-2, RealStar RT-PCR, SARS-CoV-2, RT-PCR, Ct values

## Short communication

The current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic is prompting multidisciplinary efforts towards the understanding and managing of this emerging disease. Since the outbreak, the development of reliable diagnostic assays has been one of the first goal to be addressed [1,2]. RT-PCR performed on nasopharyngeal swabs has been so far routinely used for the diagnosis.

Many RNA gene targets can be employed in the RT-PCR. Generally, 1 or more of the envelope (env), nucleocapsid (N), spike (S), RNA-dependent RNA polymerase (RdRp), and ORF1 genes are used. At present, more than 360 molecular tests are commercially available as *in vitro* diagnostic assays. Of these, 231 are Conformité Européenne (CE) marked and 69 received US emergency use authorization [3]. In this wide landscape of available diagnostic assays, the evaluation of their performance is crucial to guide the choice of an appropriate molecular test.

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This study compared the clinical performance of two commercial molecular tests for the diagnosis of SARS-CoV-2 infection: the RealStar RT-PCR kit 1.0 (Altona diagnostics, Germany) and the FTD SARS-CoV-2 Assay (Fast Track Diagnostics, Luxembourg). Both assays provide a qualitative result, and a cycle threshold (Ct) value inversely proportional to the amount of viral RNA present in tested samples.
Previous studies have described the performance of the Altona Diagnostics product compared to reference and other commercially available RT-PCR methods. For instance, a slightly better sensitivity of the RealStar® assay compared to the WHO recommended RT-PCR workflow was observed by Visseaux and colleagues [4]. In addition, Altona showed the lowest LOD95 (3.8 copies/mL) for both the E- and S-gene assays in a comparative study including other 5 RT-PCR-based methods [5].
On the other hand, since the introduction of FTD SARS-CoV-2 assay into clinical practice, no studies have evaluated its performance compared with other molecular assays.

For this purpose, 100 nasopharyngeal swabs (NPS) from people under investigation for COVID-19, collected at the Virology Unit of Umberto I University Hospital (Rome, Italy) from May 4 through June 4, 2020, were included in this study. All samples were stored at -80°C. Patients' records and information were anonymized and de-identified prior to analysis. All samples were analyzed with RealStar RT-PCR kit 1.0 (Altona-Diagnostics) and FTD SARS-CoV-2 assay (Siemens Healthineers) for the qualitative detection of SARS-CoV-2 RNA. The analysis was performed according to the manufacturer's instructions, as follows. The same nucleic acid extraction protocol was performed for both RT-PCR methods taking roughly three and a half hours. Five hundred µL of NPS were extracted using the Versant SP 1.0 kit (Siemens Healthineers) and eluted in 100 µL. Extraction was performed on Versant kPCR Molecular System (Siemens Healthineers). Both molecular assays include a heterologous amplification system (Internal Control) used as a control for extraction procedure and RT-PCR inhibition.
For the RT-PCR analysis, 10 µL of nucleic acid extract were used. Both tests are dual-target assays but with different gene targets. Amplification using RealStar RT-PCR kit 1.0 detects E and S genes with two different fluorophores and requires approximately 2 hours of processing time. Samples analysed by RealStar RT-PCR kit 1.0 (~1,5 hours for a complete run) were considered positive if at least one gene was detected. Otherwise, FTD SARS-CoV-2 test detects conserved regions within ORF1ab and N genes using a single fluorophore.
Discordant samples were tested with Xpert® Xpress SARS-CoV-2 test (Cepheid, Sunnyvale, CA, USA; LOD 0,02 PFU/mL) on the GeneXpert Dx system [6].
The agreement of the two assays was evaluated by calculating negative percent agreement (NPA), positive percent agreement (PPA), Cohen's kappa and its 95% confidence interval (CI), and by applying McNemar's test (*https://epitools.ausvet.com.au/mcnemar*). Correlation analysis was determined by using Spearman correlation coefficient. Mann-Whitney U and Bland-Altman tests were used to evaluate differences between Ct values.
The analysis of 100 NPS specimens showed that FTD SARS-CoV-2 assay reported 55 positive and 45 negative samples. In comparison, the viral RNA with RealStar RT-PCR kit 1.0 was detected in 51 specimens but not in 49 samples. The overall agreement between the two methods was 88% (88/100). Forty-seven percent (47/100) of analyzed specimens yielded a positive result for both tests, while 41% (41/100) showed a negative result. The PPA observed was 85.6% (47/55), and the NPA was 91% (41/45). Cohen's kappa coefficient was found to be 0.760 (95% CI, 0.632 to 0.887), indicating a substantial agreement between the two assays (Table 1). The McNemar test did not show a significant change in occurred proportions ($p = 0,38$). Overall, both assays did not generate false positive results.
As shown in table 1, a total of 12 discordant results were found, and their positivity was confirmed by the GeneXpert Dx system showing a median Ct value of 35,9 (IQR, 35.1-36.5).
A comparison between Ct values obtained by the two methods showed no significant difference [RealStar RT-PCR vs FTD SARS-CoV-2: median (IQR), 29.7 (23.9-34.0) vs 28.7 (24.9-34.4); $p = 0,93$] (Fig.1a). Besides, a positive correlation was observed between Ct values of positive samples ($r = 0,86$; $p < 0,001$) (Fig.1b) and a Bland-Altman plot showed a small mean difference (bias) of 0.036 ± 3.16 Ct with 95% limits of agreement ranging from -6.17 to 6.24 (Fig.1c).
Taken together, both assays were able to identify all positive samples with high viral loads and Ct values lower than 35. Discordance between test results occurred exclusively in samples with low amount of viral RNA (Ct > 35), as reported in other comparison studies [7,8].

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To the best of our knowledge, this is the first study evaluating FTD SARS-CoV-2 assay performance. The overall sensitivity is strictly dependent on the specimen's Ct values. The FTD SARS-CoV-2 assay showed a higher ability to detect positive samples. This may be due to the use of a single fluorescence channel to detect the ORF1ab and N genes simultaneously, allowing a better characterization of samples with low viral loads. On the other hand, it should be considered that measurements near the limit of detection inherently increases the stochastic noise in sampling.

In this setting, while the critical importance of analytical sensitivity, the decision to choose one assay over another should assess the balance between advantages in turn-around-time and instruments/reagents availability and the drawbacks of potentially reduced sensitivity.
In conclusion, although the limitation due to the modest sample size, this study provides valuable data about FTD SARS-CoV-2 assay and its comparison with RealStar RT-PCR kit 1.0 using clinical specimens and showing a substantial agreement between the two tests.

**Author contributions**
Daniele Di Carlo, Laura Mazzuti and Ilaria Sciandra conceived the work and wrote the text.

Giuliana Guerrizio, Giuseppe Oliveto, Rodolfo J Riveros Cabral and Maria Antonella Zingaropoli performed the testing of samples.

Ombretta Turriziani critically revised the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

**Author contributions**
Daniele Di Carlo, Laura Mazzuti and Ilaria Sciandra conceived the work and wrote the text. Giuliana Guerrizio, Giuseppe Oliveto, Rodolfo J Riveros Cabral and Maria Antonella Zingaropoli performed the testing of samples. Ombretta Turriziani critically revised the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

**Declaration of competing interest**
Di Carlo Daniele, Laura Mazzuti, Ilaria Sciandra, Giuliana Guerrizio, Giuseppe Oliveto, Rodolfo J Riveros Cabral, Maria Antonella Zingaropoli and Ombretta Turriziani have nothing to disclose.

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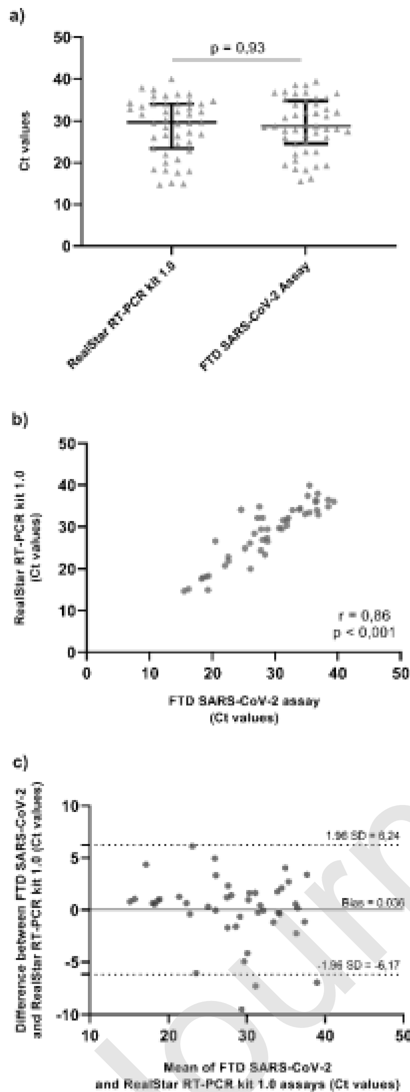


**Figure 1. (a)** Comparison of cycle threshold (Ct) values measured by RealStar RT-PCR kit 1.0 and FTD SARS-CoV-2 assays by using Mann-Whitney test; **(b)** Correlation and **(c)** Bland-Altman analysis of Ct values determined by both assays. The dotted lines represent the upper and lower limits of agreement (95% Limits of Agreement, 6.24 to -6.17) and the solid line represent the mean difference (Bias ± SD, 0.036 ± 3.16).

**Table 1.** Comparison between RT-PCR results obtained by FTD SARS-CoV-2 and RealStar RT-PCR 1.0 assays.

| **RealStar RT-PCR kit 1.0** | **FTD SARS-CoV-2 Assay** | | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |

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| | Positive | 47 | 4 | 51 |
|---|---|---|---|---|
| | Negative | 8 | 41 | 49 |
| | Total | 55 | 45 | 100 |

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