EVALUATION OF THE TALIS ONE™ COVID-19 TEST SYSTEM FOR THE RAPID DETECTION OF SARS-COV-2 AND EMERGING VARIANTS

Fall 2021

EXECUTIVE SUMMARY

Talis Biomedical Corporation (Menlo Park, CA) is transforming COVID-19 testing by providing the accuracy and precision of lab based molecular testing at the point of care with an easy-to-use rapid molecular testing solution: the Talis One™ COVID-19 Test System. It is an isothermal nucleic acid amplification test (NAAT) that detects the SARS-CoV-2 virus in under 30 minutes from nasal swab specimens. The Talis One COVID-19 Test System is designed to minimize infectivity risk to the test operator with the provided virus inactivation medium for sample collection and an entirely closed-system single-use cartridge. In a clinical study, the performance of the Talis One COVID-19 Test System showed a limit of detection (LoD) of 500 copies/mL. The analytical reactivity (inclusivity) study determined the Talis One COVID-19 Test System is expected to amplify 99.38% of published SARS-CoV-2 viral genome sequences. No cross-reactivity was observed during the analytical specificity study and the precision study confirmed within-lab and lot-to-lot repeatability. To date, Talis has completed an in silico analysis with SARS-CoV-2 genomes and performed in vitro testing of clinical samples containing specific viral genomic sequence variations. The study demonstrated equivalency in sensitivity to the comparator. The Talis One COVID-19 Test System will greatly expand access to molecular COVID-19 testing for lab-based and non-laboratory settings requiring fast, accurate, and easy-to-perform testing solutions.

INTRODUCTION

The discovery of a novel coronavirus in December 2019 in the Wuhan province of China and subsequent global spread has caused a pandemic of the scale not seen since the 1918 H1N1 pandemic. The novel coronavirus, classified as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is highly infectious and causes coronavirus disease 2019 (COVID-19), a respiratory and vascular disease associated with much higher morbidity and mortality than a seasonal influenza. During 2020, there were more than 75 million confirmed cases globally and over 1.6 million deaths.[1] The development and scaling of molecular tests for the identification of the SARS-CoV-2 virus has dramatically bolstered efforts to combat further spread of COVID-19. The initial reverse-transcriptase PCR tests developed by the World Health Organization (WHO) and the United States Centers for Disease Control and Prevention (CDC), while accurate and capable of high volume testing, could only be performed in lab settings with specialized equipment and highly-trained personnel and were associated with long turnaround times.[2] An emphasis has been placed by the U.S. government through the Rapid Acceleration of Diagnostics (RADx) Initiative to accelerate the development of rapid (< 30 minutes) COVID-19 tests capable of the same accuracy and precision as the best lab-based molecular tests that could be performed at the point of care by operators with minimal training (e.g., urgent care, etc.).[3]

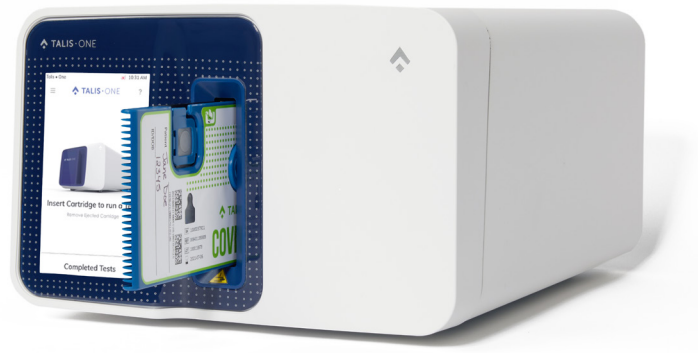


Figure 1. Talis One COVID-19 test instrument and cartridge

Talis has developed an innovative molecular diagnostic platform called the Talis One for infectious disease testing at the point of care. The system provides reliable and easy-to-perform molecular COVID-19 testing with results available in under 30 minutes. This white paper describes the design of the Talis One COVID-19 Test System and its performance across a series of studies.

MATERIALS AND METHODS

Talis One Instrument

The Talis One instrument is designed to enable rapid, highly accurate point-of-care infectious disease testing in traditional clinical microbiology and virology laboratory settings and nonlaboratory settings, including physician offices, hospital emergency departments, urgent care clinics, ambulatory surgery centers, elder care/assisted living facilities, cancer treatment clinics and dialysis centers. Each Talis One infectious disease test has a simple and streamlined workflow (Figure 2) and is intended to be used in patient care centers operating under CLIA waiver. The Talis One instrument provides intuitive and user-friendly software accessible through the embedded touchscreen for easy result interpretation and reporting.

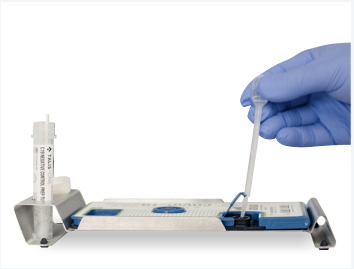
Figure 2. Test workflow for an infectious disease test on the Talis One instrument

1

Label with patient ID

2

Add specimen to cartridge



3

Insert cartridge into instrument

Results available within 30 minutes, displayed on the Talis One screen

Talis One Test Cartridge

The Talis One cartridge contains all the necessary reagents to perform a Talis One infectious disease test. Each cartridge fully automates sample lysis, nucleic acid extraction and purification, isothermal amplification, and target detection when loaded into the Talis One instrument. Specimen is added to the Talis One test cartridge through the sample port using a transfer pipette provided with each cartridge. Extraction is unique from other isothermal platforms in that the Talis One system includes an integrated solid phase DNA/RNA extraction matrix for enhanced recovery of target nucleic acid during the extraction and a purification step versus only using heat or enzymatic methods.[4] The Talis One cartridge contains five reagent wells to facilitate low-density multiplex test designs. Once testing is complete, the Talis One cartridge can be disposed of in biohazardous waste.

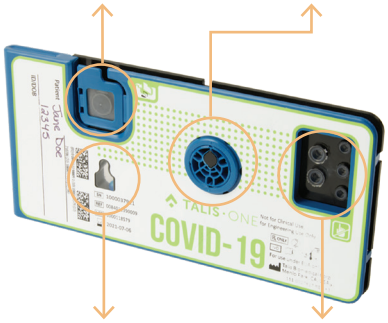
Talis One COVID-19 Test System

COVID-19 is the first infectious disease test on the Talis One instrument. This qualitative, ~27-minute rt-RT-LAMP (real-time, reverse transcription loop-mediated isothermal amplification)-based test, targets the ORF1ab and the N gene of the SARS-CoV-2 virus, and includes an on-board sample processing control targeting human beta-actin (HBA) RNA.

Preliminary *in silico* analysis suggest the Talis One COVID-19 test will not be impacted by the United Kingdom strain B.1.1.7, and up to 10 other variants. A small internal study conducted separately in clinical specimens showed correct identification of the Delta variant. The Talis One COVID-19 Test is compatible with nasal swab specimens collected in the Talis One Nasal Collection Kit. Each test kit includes a Talis One COVID-19 Test cartridge and 1 mL disposable pipette for transfer of collected sample to the sample port. The Talis One Nasal Collection Kit includes a 10 mL polypropylene tube with 3 mL of medium and a swab with pre-scored breakpoint for specimen collection. Approximately 1 mL of sample is transferred into the cartridge for testing.

Figure 3. The Talis One COVID-19 Test Cartridge

- Open sample port: wide range of sample input volume
- Solid phase DNA/RNA extraction (configurable by changing rotor)



- Sample metering and measurement
- Primers and probes in independent wells

RESULTS

Clinical Study[4]

Clinical performance of the Talis One COVID-19 test was assessed by comparison to an FDA authorized molecular SARS-CoV-2 assay (EUA). A total of 82 clinical nasal swab specimens previously collected from individuals suspected of COVID-19 by their healthcare provider, or previously diagnosed with COVID-19, were tested with both the Talis One COVID-19 Test System and the comparator test. Specimens were tested at two external point-of-care sites in a blinded and randomized fashion.

Table 1. Clinical Performance Study Results of the Talis One COVID-19 Test System

		FDA Authorized Molecular SARS-CoV-2 Test (EUA)		
		Positive	Negative	Total
Talis One COVID-19 Test System	Positive	38	0	38
	Negative	0	39	39
	Total	38	39	77
PPA		100% (95% CI 90.8%–100%)		
NPA		100% (95% CI 91.0%–100%)		

The Talis One COVID-19 Test System had a positive percent agreement (PPA) of 100% (95% CI: 90.8%-100%) and a negative percent agreement (NPA) of 100% (95% CI: 91.0%-100%).

Analytical Sensitivity Study

An analytical sensitivity (LoD) study was performed using the BEI Resources SARS-Related Coronavirus 2, Isolate USA-WA1/2020 (gamma-irradiated), which was titered into Talis One Nasal Collection Medium to create the LoD samples. Samples were tested in replicates of three during the initial range-finding. Subsequently, LOD was verified by 20 replicates at a concentration which gave 95% or higher positive frequency. The LoD of the Talis One COVID-19 test was determined to be 500 copies/mL.

Table 2. LoD Confirmation Results, SARS-CoV-2

Sample Concentration (copies/mL)	Cartridge Lot	Positive Call Frequency	Percent Frequency
125	Lot 1	19/20	95%
250	Lot 1	20/20	100%
250	Lot 2	18/20	90%
500	Lot 2	20/20	100%

Analytical Specificity Study

An analytical specificity study was performed both by *in silico* analysis and *in vitro* testing. During the *in silico* analysis, none of the 30 organisms tested were predicted to cross-react with the primers and probes of the Talis One COVID-19 test. For *in vitro* testing, serial dilutions of potentially cross-reactive organisms were spiked into pooled negative nasal swab specimen matrix and then three replicated were tested for each organism. No cross-reactivity was observed for any organism at the concentration tested (Table 3).

Analytical Reactivity (Inclusivity)

Analytical reactivity of the Talis One COVID-19 Test System was evaluated by in silico analysis of the assay primers and probes in relation to nucleotide sequences available from the NCBI database as of July 21, 2021. Overall, the Talis One COVID-19 Test System is expected to amplify 171,513/172,577 or 99.38% of published sequences.

As 0.62% of sequences were not a perfect match in both targets (gene N and gene ORF1ab), assay performance will continue to be monitored and tested to assess the potential for false negatives if applicable.

SARS-CoV-2 Variant Monitoring/Testing

An *in silico* analysis of 589,887 US sequences from the GISAID database (accessed July 22, 2021) was performed to evaluate the impact of emerging viral mutations on assay performance. The prevalence of variants within the primer/probe regions of the Talis One COVID-19 test targets was below the 5% total population threshold established by the FDA guidance with the exception of one single nucleotide polymorphism (SNP)—G29402T—which had 39,705 counts for a prevalence of 6.8%. The next most prevalent SNP within the primer/probe regions had 2,144 counts for a total prevalence of less than 1%. All other SNPs within the Talis One COVID-19 test primer/probe regions were present in less than 1% of sequences added in the past 60 days (from the date of testing, July 22, 2021).

Clinical sample testing of SARS-CoV-2 variants with the Talis One COVID-19 test was performed as a part of the RADx Variant Task Force (an NIH project), a program implemented to assess the impact of variants of concern (VOC) and/or variants of interest (VOI) circulating in the United States on RADx-funded tests. Blinded testing of the variant sample pools was performed at Talis in triplicates. The following variants were included: B.1.2, B.1.1.7, B.1.351, B.1.375, B.1.427, B.1.429, B.1.525, B.1.526, P1, and P2. Positive and negative controls were run at the beginning of each study testing day according to the Talis One COVID-19 Test System Instructions for Use. The blinded panel contained different concentrations of the VOC or VOI, an inactivated positive and

Table 3. *In vitro* Cross-Reactivity Study Results

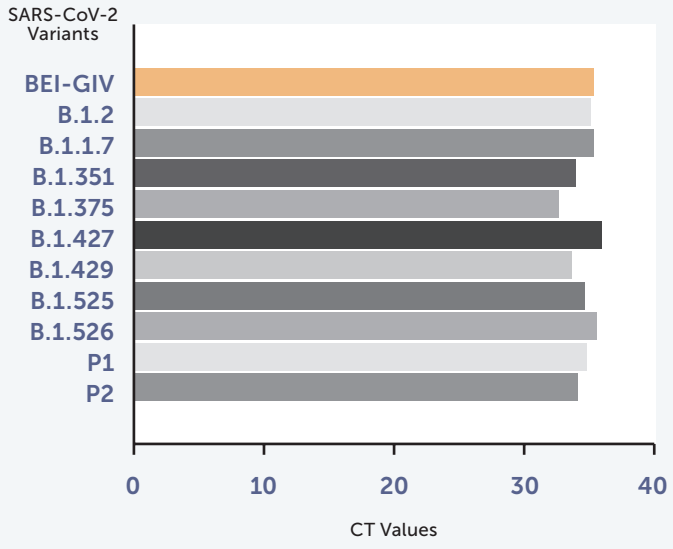
Organism Name	Testing Concentration	Frequency Detected (%) at Testing Concentration
Microorganisms from the same genetic family		
Coronavirus 229E	4.17×10^4 $TCID_{50}$/mL	0.0%
Coronavirus 0C43	1.26×10^5 $TCID_{50}$/mL	0.0%
Coronavirus HKU1	1×10^5 genome copies/mL	0.0%
Coronavirus NL63	1.41×10^4 $TCID^{50}$/mL	0.0%
SARS-coronavirus	Ct 25-28[5]	0.0%
MERS-coronavirus	3.55×10^4 $TCID_{50}$/mL	0.0%
High priority organisms		
Adenovirus	1×10^6 $TCID_{50}$/mL	0.0%
Mycoplasma pneumoniae	1×10^6 CCU/mL	0.0%
Human Metapneumovirus	1.55×10^3 $TCID_{50}$/mL	0.0%
Parainfluenza Virus 1	5.01×10^4 $TCID_{50}$/mL	0.0%
Parainfluenza Virus 2	1.51×10^5 $TCID_{50}$/mL	0.0%
Parainfluenza Virus 3	1×10^6 $TCID_{50}$/mL	0.0%
Parainfluenza Virus 4	1.38×10^6 $TCID_{50}$/mL	0.0%
Influenza A	1.41×10^4 $TCID_{50}$/mL	0.0%
Influenza B	1.17×10^4 $TCID_{50}$/mL	0.0%
Enterovirus	1.26×10^5 $TCID_{50}$/mL	0.0%
Respiratory syncytial virus	1.26×10^5 $TCID_{50}$/mL	0.0%
Rhinovirus	1.26×10^5 $TCID_{50}$/mL	0.0%
C. pneumoniae	1×10^6 $TCID_{50}$/mL	0.0%
H. influenzae	1×10^6 $TCID_{50}$/mL	0.0%
L. pneumophila	1×10^6 $TCID_{50}$/mL	0.0%
M. tuberculosis	1×10^5 genome copies/mL	0.0%
S. pneumoniae	1×10^6 $TCID_{50}$/mL	0.0%
S. pyogenes	1×10^6 $TCID_{50}$/mL	0.0%
B. pertussis	1×10^6 $TCID_{50}$/mL	0.0%
C. albicans	1×10^6 $TCID_{50}$/mL	0.0%
P. aeruginosa	1×10^6 $TCID_{50}$/mL	0.0%
S. epidermidis	1×10^6 $TCID_{50}$/mL	0.0%
S. salivarius	1×10^6 $TCID_{50}$/mL	0.0%
Pooled human nasal wash	10%	0.0%
P. jirovecii	1×10^6 nuclei/mL	0.0%

one negative control (56 blinded samples plus an additional negative matrix control).

Results were reviewed and analyzed by RADx personnel and results were provided to Talis by the RADx Variant Task Force. The Ct values obtained for the variants at the N2 QC were compared to the Ct values obtained for the BEI-GIV and the B.1.2 strains at initial QC with the CDC N2 target.

Test results were either positive (+), negative (-), or invalid (I). A positive result was recorded if two or three of three replicates from a sample pool were positive. A negative result was recorded if less than two replicates from a pool were positive. The dilution level with the lowest viral load (highest Ct at the N2 QC) recorded as positive was considered the detection level (see Figure 4). The negative controls (blinded and unblinded) as well as the positive ones showed the expected negative and positive results, respectively. The tested cohort included five randomly distributed invalid results that were not repeated. The lowest dilution (Dil E) of the P1 variant that was tested was not detected. Otherwise, all variants were detected in all three replicates of each dilution including the highest dilution. Note that RADx personnel made panels with Ct targets going up to a certain level and that dilutions were not titrated until failure. At the time of testing in June 2021, the emerging Delta variant was not available to be included in this study. Subsequently, a small internal study showed correct identification of the delta variant in clinical specimens.

Figure 4. Comparison of VOC/VOI with wild type, BEI-GIV shows equivalency in sensitivity.



DISCUSSION

At the onset of the COVID-19 pandemic in the winter of 2020, the global spread of SARS-CoV-2 virus greatly outpaced the capacity to test for the virus. Real-time reverse transcription PCR (rt-RT-PCR) became the initial workhorse for COVID-19 testing because of the ease and quickness to develop an effective assay and the ability to leverage the massive installed base of real-time PCR instruments globally. These assays served and continue to serve a tremendous purpose with their ability to process extremely high volumes of patient samples to provide visibility to the location and spread of the SARS-CoV-2 virus. However, these tests can only be performed in specialized lab settings with highly trained personnel. The tests themselves also have long turnaround times (at least 3-4 hours) often taking multiple days to be reported based on the backlog of testing the laboratories performing this testing are experiencing, limiting their utility.

Rapid, molecular point of care (POC) testing is emerging as a favored approach for COVID-19 testing in the United States. Since the launch of the first molecular POC tests from Abbott, Roche, and Cepheid, market demand has been extraordinarily high for these tests. And, performance of these molecular POC tests has not been identical, because of different features which influence genetic target extraction, purification and ultimately test sensitivity. The Talis One COVID-19 test is unique from existing isothermal assays because of the integration of solid phase extraction and purification, as demonstrated by the LoD observed in this study. Additional testing using the FDA SARS-CoV-2 Reference Panel will be required to exactly pinpoint the Talis One COVID-19 test LoD relative to the other molecular POC tests and high-throughput assays.

CONCLUSION

Expansion of SARS-CoV-2 testing in near-patient and workplace settings will help accelerate the fight against further spread of COVID-19. While Talis continues to study the impact of performance of variants, preliminary data show that the Talis One COVID-19 Test is an accurate and precise molecular diagnostic assay for the detection of common circulating variants of the SARS-CoV-2 virus from nasal swab specimens in under 30 minutes and has the ability to quickly become one of the leading POC assays for COVID-19 testing.

1. COVID-19 Weekly Epidemiological Update. https://covid19.who.int/ Accessed September 2021
2. WHO lists two COVID-19 tests for emergency use. https://www.who.int/news/item/07-04-2020-who-lists-two-covid-19-tests-for-emergency-use. Accessed January 4, 2021
3. National Institutes of Health: Rapid Acceleration of Diagnostics (RADx). https://www.nih.gov/research-training/medical-research-initiatives/radx. Accessed January 4, 2021
4. Talis COVID-19 Test System IFU Rev X1 (redline 152-0028851 Rev. X1)

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