EVALUATION OF THE TALIS ONE™ COVID-19 ASSAY FOR THE RAPID DETECTION (< 30 MINUTES) OF SARS-COV-2 FROM NASAL SWAB SPECIMENS

TALIS

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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 a compact, low cost and easy-to-use rapid molecular testing solution: the Talis One™ System. The Talis One COVID-19 Assay is an isothermal nucleic acid amplification test (NAAT) that detects the SARS-CoV-2 virus in under 30 minutes from nasal swab specimens collected in the Talis One Inactivation Media. The Talis One COVID-19 Assay is designed to minimize infectivity risk to the test operator with virus inactivation media for sample collection and an entirely closed-system single-use test cartridge. In the accuracy study, a series of analytical tests were conducted to characterize the performance of the Talis One COVID-19 Assay and three hundred residual clinical samples were included. The Talis One COVID-19 Assay had a positive and negative percent agreement of 97.0% and 99.0%, respectively. The analytical sensitivity (limit of detection; LOD) was determined to be 500 copies/mL. The analytical reactivity (inclusivity) study determined the Talis One COVID-19 Assay detects one hundred percent of the 436 published SARS-CoV-2 sequences. No cross reactivity was observed during the analytical specificity study and the precession study proved the Talis One COVID-19 Assay to have within-lab and lot-to-lot precision. The Talis One System and the Talis One COVID-19 Assay 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.

Figure 1. Two stacked Talis One systems and COVID-19 test cartridges

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), 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 1.5 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 real-time 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 (RADxSM) 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 (POC) by operators with minimal training (e.g., urgent care, etc.).[3]

Talis Biomedical ("Talis") has developed an innovative molecular diagnostic platform called the Talis One System for infectious disease testing at the point-of-care. The Talis One System and the Talis One COVID-19 Assay 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 Assay and its performance across a series of analytical studies.

Figure 2. Test workflow for an infectious disease assay on the Talis One System

1. Add patient ID: barcode or handwritten
2. Transfer specimen sample with provided transfer pipette
3. Add sample to Talis One cartridge
4. Insert test cartridge into the Talis One
5. Results available within 30 minutes, displayed on the Talis One screen

MATERIALS AND METHODS

Talis One System

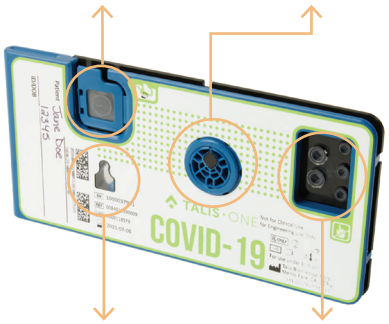
The Talis One system is a compact, sample-to-answer molecular diagnostics solution designed to enable rapid, highly accurate point-of-care infectious disease testing in traditional clinical microbiology and virology laboratory settings and nonlaboratory settings, including physician offices, hospital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

Talis One Test Cartridge

The Talis One test cartridge is a fully self-contained, closed system that contains all the necessary reagents to perform a Talis One infectious disease assay. Each cartridge fully automates sample lysis, nucleic acid extraction and purification, isothermal amplification, and target detection when loaded into Talis One. Specimen is added to the Talis One test cartridge through the sample port using the exact volume transfer pipette provided with each Talis One infectious disease assay. Extraction on the Talis One System 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 purification step versus only using heat or enzymatic methods.[4] The Talis One test cartridge is also designed for flexible multiplexing, containing five reagent wells to facilitate low-density multiplex assay designs. Once testing is complete, the Talis One test cartridge can be disposed of in biohazard waste.

Figure 3. The Talis One COVID-19 Assay 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

Talis One COVID-19 Assay

The Talis One COVID-19 Assay is the first infectious disease assay in the Talis One system. This qualitative, ~27-minute rtRT-LAMP (real-time reverse transcription Loop-Mediated Isothermal Amplification) based assay targets the *ORF1ab* gene and the *N* protein gene of the SARS-CoV-2 virus and includes an on-board sample processing control with the human beta-actin (HBA) target. Preliminary *in silico* analysis suggest the Talis One COVID- 19 Assay will not be impacted by the United Kingdom strain B.1.1.7. The Talis One COVID-19 Assay is compatible with nasal swab specimens collected in the Talis One Inactivation Media. Each test kit includes a Talis One COVID-19 Assay cartridge and individually packed 1 mL disposable transfer pipette for transfer of collected sample to the cartridge. The Talis One Nasal Specimen Inactivation Kit includes a 10 mL PP tube with 3 mL of Talis One Inactivation Media and an individually packaged flocked nasal swab with pre-scored breakpoint for specimen collection. Approximately 1 mL of sample is added by the user into the Talis One COVID-19 Assay cartridge for testing using the transfer pipette and ~950µL is metered out within the cartridge for testing.

Analytical Studies

A series of studies were conducted to characterize the performance of the Talis One COVID-19 Assay on the Talis One system, including an accuracy study, analytical sensitivity (LOD), analytical reactivity (inclusivity), analytical specificity (exclusivity), and precision study. These studies were conducted at the Talis laboratory in Menlo Park, California.

RESULTS

Accuracy Study

A total of 300 residual clinical specimens were tested with the Talis One COVID-19 Assay during the accuracy study. All Talis One testing was performed at the Talis headquarters in Menlo Park, CA. The deidentified residual samples were provided frozen to Talis by Fulgent Therapeutics, LLC, and consisted of anterior, mid-turbinate nasal swab specimens collected in the in the DNA/RNA Shield inactivation buffer (Zymo Research). These samples were first tested with the Fulgent COVID-19 by RT-PCR Test. For the Talis One workflow, samples were thawed and spun down prior to testing. Discrepant analysis, when required, was performed with the *Quick* SARS-CoV-2 rRT-PCR assay from Zymo Research on the BioRad CFx96 at Talis. The Talis One COVID-19 Assay had a positive and negative percent agreement of 97.0% and 99.0%, respectively. Following discrepant testing, the one apparent false positive Talis One result tested as negative with the Zymo assay and three of the six apparent false negative Talis One results tested as negative with the Zymo assay.

Table 1. Performance of the Talis One COVID-19 Assay in the Accuracy Study

		FDA Authorized Molecular SARS-CoV-2 Assay (EUA)[a]		
		Positive	Negative	Total
Talis One COVID-19 Assay	Positive	194	1[b]	195
	Negative	6[c]	99	105
	Total	200	100	300
PPA		97.0% (93.1% - 98.8%)		
NPA		99.0% (93.4% - 99.9%)		

a. Specimens were frozen and spun prior to testing
b. One specimen tested negative by Zymo Quick SARS-CoV-2 rRT-PCR Kit
c. Three specimens tested negative and three positive by Zymo Quick SARS-CoV-2 rRT-PCR kits

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 Inactivation Media to create the LOD samples. Samples were tested in three replicates at multiple concentrations of a dilution series until the assay had less than 100% agreement for a given concentration. Subsequently, 20 replicates at the final concentration with 100% correlation were tested, with a minimum of 95% (19/20) correlation required to establish LOD. The LOD of the Talis One COVID-19 Assay was determined to be 500 copies/mL.

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 27 organisms tested were predicted to cross-react with the primers and probes of the Talis One COVID-19 Assay. 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 2).

Table 2. *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%
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%

Analytical Specificity Study

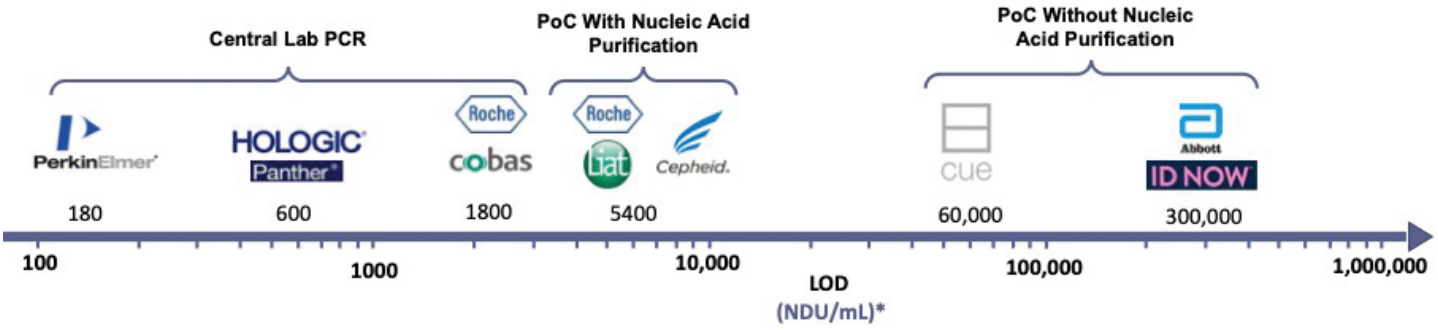
Studies were performed to confirm within-lab and lot-to-lot precision of the Talis One COVID-19 Assay. Sample type, concentration, Talis One instrument, and test operators were varied across the studies to test assay precision. A total of 396 tests were performed and the Talis One COVID-19 Assay had 99.8% (395/396) correlation across these studies.

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 (rRT-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 install 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 test themselves also have long turnaround times (at least 3-4 hours) often taking multiple days to weeks to be reported based on the backlog of testing the laboratories performing this testing are experiencing, limiting their utility.

Rapid, molecular 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 none of these manufacturers have been able to build up sufficient scale to come close to meeting demand. Performance of these molecular POC tests have not been identical, however. The LOD of the real-time PCR (qPCR) based Roche Liat SARS-CoV-2 & Influenza A/B (Roche, Basel, Switzerland) and the Cepheid GeneXpert Xpress Sars-CoV-2 (Danaher, Washington, D.C.) have greatly exceeded that of the isothermal based assays, including Abbott ID Now COVID-19 Assay (Abbott Laboratories, Abbott Park, IL) and Cue COVID-19 test (Cue Health, San Diego, CA). The Liat and Xpert Xpress assays begin to approach the LOD of the high-throughput lab-based SARS-CoV-2 assays noted for best-in-class analytical sensitivity (Figure 4).[7] This superior LOD has translated into improved detection of SARS-CoV-2 virus for the Liat and Xpert Xpress assays when compared to the Abbott ID Now assay, resulting in better positive percent agreement in these studies.[4,7] The Talis One COVID-19 Assay is unique from existing isothermal assays like ID Now 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 Assay LOD relative to the other molecular POC tests and high-throughput assays.

Figure 4. LOD determined using the FDA SARS-CoV-2 Reference Panel



CONCLUSION

Expansion of SARS-CoV-2 testing in near-patient and workplace settings will help accelerate the fight against further spread of COVID-19. The Talis One COVID-19 Assay is an accurate and precise molecular diagnostics assay for the detection of the SARS-CoV-2 virus from swab specimens in under 30 minutes and is poised to quickly become one of the leading POC assays for COVID-19 testing.

Acknowledgements

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