Preclinical Validation of a Rapid Molecular Test for Point-of-Care Detection of *Treponema pallidum* for Early Syphilis Diagnosis

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Abstract

Reported cases of syphilis in the United States increased by 42% from 2020 to 2024, with congenital syphilis rising 700% over the past decade.[1] Current diagnostics rely on serological testing that often requires multiple reflex assays, frequently misses primary infection (14-46% of cases[2]), and lacks a timely diagnosis needed to prevent loss to follow-up during early, highly transmissible stages. Tetracore, Inc. has developed a real-time PCR assay [the *T. pallidum* (Syphilis) test] that directly detects *T. pallidum* from lesions and rashes present during primary and secondary syphilis. The test is optimized for room temperature stability and does not require DNA extraction (e.g., samples are tested directly) or pipetting and centrifugation. The *T. pallidum* (Syphilis) test is run on the portable T-COR 8™ real-time PCR thermocycler in under 45 minutes.

Analytical sensitivity, specificity, stability, reproducibility, and repeatability testing was previously completed. This led to the design and conduct of a preclinical validation study, using contrived clinical samples generated by spiking live *T. pallidum* organism into syphilis-negative clinical matrices. Swab specimens from vaginal, rectal, penile, dermal, and oral lesions were obtained, and syphilis-negative status was confirmed by PCR. The *T. pallidum* (Syphilis) test was evaluated using the two primary clinically relevant lineages of *T. pallidum* ssp. *pallidum*, SS14 and Nichols. Preclinical sensitivity testing demonstrated 95% limits of detection (LoD) ranging from 1.1 – 2.2 x 10^3 treponemes/mL, determined using linear Probit regression analysis, with consistent repeatability across 40 replicates spanning multiple production lots. High microbial load and carryover testing was also conducted, first tested at high *T. pallidum* concentrations (up to 2.7 x 10^{11} genomic copies/mL) across multiple runs, followed by multiple runs of negative samples; no false positives were observed.

Ongoing studies include inclusivity across 9 live *T. pallidum* strains, and cross-reactivity and interference testing with 66 microbial species and 22 exogenous agents using contrived samples. Storage stability has been demonstrated at 25 °C, 37.5 °C, and 55 °C. The *T. pallidum* (Syphilis) test is being advanced toward FDA 510(k) clearance and CLIA waiver, with the potential to significantly improve early syphilis detection and patient outcomes.

Introduction

Syphilis is a STI with rising incidence. There is a significant unmet need for its improved diagnosis. Incorporating IVD assays into the POC setting may enable earlier detection before patients are lost to follow-up, particularly during early infection when transmission and disease progression risk is highest. The *Treponema pallidum* (Syphilis) Test is a CLIA-waivable POC test designed for use with Tetracore's T-COR 8™ system.

- The **Sample Collection Device** enables direct testing from lesion exudate and other clinical specimens. Breaking the snap-valve releases a buffer that resuspends the sample, preparing it for direct PCR testing with no need for extraction.
- The **Collect-to-Test (C2T) Cartridge** is a self-metering reaction device with a sample reservoir that accommodates variable fluid volumes. A plunger transfers sample into a reaction chamber containing dried, room temperature-stable PCR reagents, eliminating the need for pipetting.
- The **T-COR 8™** is a portable multi-sample real-time thermocycler that operates on internal battery power and weighs under 10 lbs. It is suitable for use in clinics or physician offices and is CE-IVD. A proprietary algorithm (SmartCT™) analyzes and interprets results across each of the channels to provide a *Detected*/*Not Detected* call.

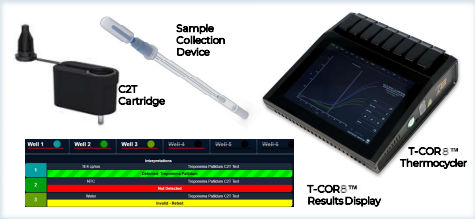

Figure 1. The Tetracore T-COR 8 system.

Methods

Multiplexed Assay Design

- Two real-time PCR assays target different regions of the highly homologous *polA* gene; both assays detected in FAM channel
- A synthetic internal control (IC) is detected in HEX channel

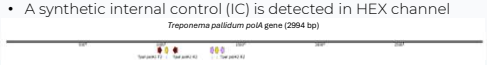

Figure 2. Design of the two assays targeting the *polA* gene of *T. pallidum*.

Lyophilization of *T. pallidum* (Syphilis) Tests:

- 11 lots (N=1560) of *T. pallidum* (Syphilis) Tests produced and QC-tested; all lots passed and were used for validation
- Performed under an ISO 9001/13485 QMS in preparation for accreditation

Preparation of Clinical Samples:

- Patient swabs from vaginal, rectal, penile, sacral, pubic, and oral lesions were collected under an IRB and confirmed negative for *T. pallidum* by PCR

Figure 3. Overview of preclinical analytical validation study.

Results

Preclinical Sensitivity

Limit of Detection:

- 95% LoD (via linear Probit regression) determined across the two clinically-relevant syphilis lineages:
  - **Nichols:** 2189.6 Tp/mL (82.11 Tp/reaction)
  - **SS14:** 1076.5 Tp/mL (40.37 Tp/reaction)
- Assay LoD is **3 logs lower** than clinically-relevant concentrations reported in human syphilis lesions (~5x10^6 copies/mL)[3]

Repeatability:

- Evaluated multiple production lots across both lineages at 3x LoD in contrived clinical samples
- **All 56 replicates (100%) successfully detected *T. pallidum***

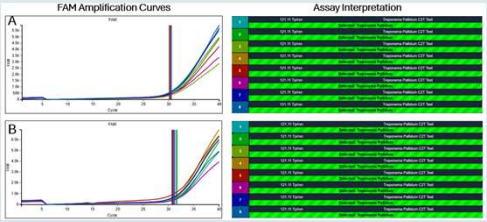

Figure 4. Example replicates from lots (A) 101525 and (B) 100625, tested at 3x LoD SS14 on the same T-COR 8 by the same technician.

High Microbial Load & Carry-Over Testing:

- **Assay detected 100%** of high *T. pallidum polA* concentrations tested, with **no carry-over contamination observed**

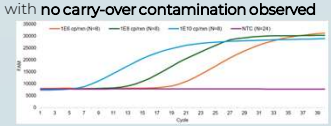

Figure 5. Median FAM fluorescence values at high *T. pallidum* DNA concentrations followed by NTCs; all run on the same T-COR 8 on the same day.

Results (cont.)

Preclinical Specificity

Inclusivity Testing:

- **Similar sensitivity across 9 syphilis strains from both lineages**, including circulating strains from the U.S. and Western Europe

Table 1. Detection of various *T. pallidum* ssp. *pallidum* strains.

Strain Name	Collection Date	Lineage	Concentration Tested	Replicates, N	"Tpal Detected", N (%)
Haiti B	1951	SS14	1x LoD: 1.08E3 Tp/mL	10	9 (90)
			2x LoD: 2.15E3 Tp/mL	3	3 (100)
MexicoA	1953	SS14	1x LoD: 1.08E3 Tp/mL	3	3 (100)
			2x LoD: 2.15E3 Tp/mL	3	3 (100)
Sea 81-4	1981	Nichols	1x LoD: 2.19E3 Tp/mL	3	3 (100)
			2x LoD: 4.38E3 Tp/mL	3	3 (100)
UW231	4/22/2004	SS14	1x LoD: 1.08E3 Tp/mL	3	3 (100)
			2x LoD: 2.15E3 Tp/mL	3	3 (100)
UW244	6/22/2004	SS14	1x LoD: 1.08E3 Tp/mL	3	3 (100)
			2x LoD: 2.15E3 Tp/mL	3	3 (100)
UW259	8/5/2004	SS14	1x LoD: 1.08E3 Tp/mL	3	3 (100)
			2x LoD: 2.15E3 Tp/mL	3	3 (100)
UW279	10/14/2004	Nichols	1x LoD: 2.19E3 Tp/mL	3	3 (100)
			2x LoD: 4.38E3 Tp/mL	3	3 (100)
UW383	11/9/2005	SS14	1x LoD: 1.08E3 Tp/mL	3	3 (100)
			2x LoD: 2.15E3 Tp/mL	3	3 (100)
UW526	6/15/2007	SS14	1x LoD: 1.08E3 Tp/mL	3	3 (100)
			2x LoD: 2.15E3 Tp/mL	3	3 (100)

In Silico *Mismatch Analysis & Cross-Reactivity:*

- BLAST analysis of all primers and probes **confirmed 100% detection of all *T. pallidum* ssp. *pallidum* sequences** in NCBI GenBank
- BLAST analysis revealed cross-reactivity against *T. pallidum* near neighbors Yaws and Bejel; **both are distinguishable from venereal syphilis[4] and extremely rare in the U.S.**

Table 2. Reactivity of closely related *T. pallidum* near neighbor strains.

Organism	Clinical Significance	Percent Match (*In Silico*)	Bench Testing Results
T. pallidum ssp. *pertenue* Samoa D	Causes Yaws: nonvenereal, endemic disease (rare in U.S.)	99.70%	*T. Pallidum* **detected** in 3/3 samples with 1E5 CFU/reaction *T. pallidum* ssp. *pertenue* Samoa D
T. pallidum ssp. *endemicum** Bosnia A	Causes Bejel: nonvenereal, endemic disease (rare in U.S.)	99.70%	*T. Pallidum* **detected** in 3/3 samples with 1E5 CFU/reaction *T. pallidum* ssp. *endemicum* Bosnia A
Treponema paraluiscuniculi Cuniculi A	Causes venereal spirochetosis in rabbits ("rabbit syphilis")[5]	99.40%	*T. Pallidum* **detected** in 3/3 samples with 1E5 CFU/reaction *T. paraluiscuniculi* Cuni A
Treponema phagedenis Reiter	Non-pathogenic[6]	65.26%	*T. pallidum* was **not detected** in 3/3 samples with 1E5 CFU/reaction *T. phagedenis*

*Matched multiple *T. pallidum pertenue* and *endemicum* strains *in silico*, 1 strain from each species empirically tested for confirmation.

Microbial Interference:

- **No interference or cross-reactivity observed across microbes** tested at ≥10^5 CFU/PFU per test in contrived clinical specimens with and without *T. pallidum*

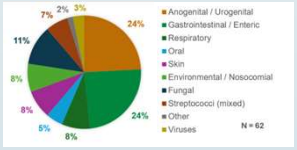

Figure 6. Composition of specificity panel evaluated ± *T. pallidum* spiked into sample.

Exogenous Agent Interference:

- Multiple clinically relevant substances were shown to be **non-inhibitory at clinically relevant concentrations**

Table 3. Non-inhibitory concentrations of exogenous agents.

Non-inhibitory Concentration	Exogenous Agents
10% v/v	Urine, Douche
2.5%v/v	Dermoplast*, Seminal Fluid*
1% v/v	Betadine*
0.25%v/v	Whole Blood*
0.05%v/v	Fecal Matter*
10 mg/mL	Contraceptive gel, Erythromycin*, Neosporin, Preparation H, Hygenic Cleansing solution (Baineol), Vaginal Antifungal ointment, K-Y Jelly, Monistat 3
5 mg/mL	Cidofovir*, CeraVe*
1 mg/mL	Acyclovir*, Vagisil*
0. 1 mg/mL	Human albumin serum*
1E5 cells/reaction	Leukocytes

*Highest concentration tested that was non-inhibitory to the *T. pallidum* (Syphilis) test.

Results (cont.)

Preclinical Environmental Effects:

Table 4. Summary of preclinical environmental effects experiments.

Assessment	Test Condition	*T. pallidum* Detection Results at 3x LoD	Observed Impacts
Sample stability	1 week storage of samples in collection devices at 4°C and 25°C	100% detection (12/12) at 1 week time-point at 4°C and 25°C	Loss of sample over time
High humidity	1 week storage at 80% Rh, 15°C	100% detection (10/10) at 1 week time-point	None
Extreme temperature cycling	Cycling between -20°C and 37.5°C every 24 hours for 1 week	100% detection (10/10) at 1 week time-point	None
Vibration testing	120 rpm for 2 hours in normal and upside-down orientation	100% detection (20/20)	None
Drop testing	Dropped 10 times from height of 1.2 meters	100% detection (10/10)	None
Storage stability	Long-term storage at 55°C, 37.5°C, and 25°C	100% detection up to 1 week at 55°C (3/3 detected at 3x LoD, 3/3 detected at 10x LoD) and 100% detection up to 3 months at 37.5°C (on-going) and 14 months at 25 °C (on-going)	None

Conclusions

The *T. pallidum* (Syphilis) Test demonstrated:

- **Robust detection with clinically relevant LoD** (2189.6 Tp/mL [Nichols] and 1076.5 Tp/mL [SS14])
- **Detection of contemporary and historical** *T. pallidum* ssp. *pallidum* strains from SS14 and Nichols lineages
- **No interference at high concentrations of 62 microbial organisms** (bacteria, viruses, and fungi)
- **No interference of 22 exogenous agents** at various clinically relevant concentrations
- Detection of 2 *T. pallidum* near neighbors; both cause non-venereal, endemic diseases (rare in the U.S.) that are **clinically and geographically distinct from syphilis** (Yaws, Bejel)
- **Repeatability** across production lots and *T. pallidum* lineages
- **Stability in various environmental conditions**, including high humidity, temperature cycling, mechanical stresses, and time; currently stable at room temperature for up to **14 months** (data not shown)

Plan Forward

- ***Clinical Validation Studies:*** Evaluation of 30 syphilis-positive and 30 syphilis-negative clinical specimens using the *T. pallidum* (Syphilis) Test; results will be directly compared to a CLIA laboratory test
- ***Regulatory Plan:*** Clinical study and 510(k) with CLIA waiver submission to the FDA

Acknowledgments

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Abbreviations: C2T, Collect-to-Test; CE-IVD, Conformité Européenne – In Vitro Diagnostic; CFU, colony forming units; CLIA, Clinical Laboratory Improvement Amendments; cp, DNA copies; FDA, Food and Drug Administration; IC, internal control; IRB, Institutional Review Board; ISO, International Organization for Standardization; IVD, *in vitro* diagnostic; LoD, limit of detection; NCBI, National Center for Biotechnology Information; NTC, no template control; PCR, polymerase chain reaction; PFU, plaque forming units; POC, point-of-care; QC, quality control; QMS, quality management system; rxn, reaction; STI, sexually transmitted infection; Tp, treponemes.