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Evaluation of the GeneSoC rapid quantitative PCR system for *Treponema pallidum* detection

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ABSTRACT

The incidence of syphilis, caused by *Treponema pallidum* (TP), is increasing worldwide. Nucleic acid amplification tests, including quantitative PCR (qPCR), are valuable for diagnosing primary syphilis, particularly using ulcer/lesion swabs. Recent studies have also shown the promising diagnostic performance of nucleic acid amplification tests using saliva. The GeneSoC platform, a rapid qPCR system capable of completing 50 PCR cycles in 15 min, has been used for the diagnosis of infectious diseases, but has not been assessed for syphilis. This study aimed to evaluate the performance of the GeneSoC rapid qPCR assay for TP detection in clinical samples. We evaluated clinical specimens from ulcer/lesion swabs (syphilis, $n = 43$; non-syphilis, $n = 20$) and saliva (syphilis, $n = 33$; non-syphilis, $n = 20$). All syphilis samples were confirmed to be positive by conventional qPCR and stored before analysis with the GeneSoC rapid qPCR assay. The GeneSoC rapid qPCR assay had a detection limit for TP DNA of 20 copies/reaction, compared with 2 copies for the conventional qPCR assay. The results for the GeneSoC and conventional qPCR assays showed 100 % concordance for ulcer/lesion swabs. For saliva samples, the positive agreement rate was lower with crude DNA (63.6 %) but improved with purified DNA (84.8 %). The negative agreement rate was 100 % for both sample types. The GeneSoC rapid qPCR assay is a promising point-of-care test for primary syphilis using ulcer/lesion swabs. However, further optimization and validation, especially for saliva, are needed for its broader clinical use.

Syphilis is a sexually transmitted disease caused by *Treponema pallidum* (TP), and its incidence has increased significantly worldwide in the past few decades [1]. To prevent the transmission of syphilis, it is necessary to identify infected individuals through appropriate diagnostic tests and the treatment of positive cases. Syphilis is classified into several clinical stages: primary, secondary, latent, and tertiary. Syphilis can also present as neurosyphilis, ocular syphilis, and otosyphilis. There are several challenges in diagnosing syphilis. Since TP is difficult to culture, the diagnosis of syphilis is based on treponemal and non-treponemal serological tests. Patients are often asymptomatic, so it is necessary to recommend testing for individuals at risk of infection. Furthermore, it takes 2–4 weeks after infection for antibodies to become detectable in the bloodstream, which means that patients with early-stage primary syphilis (i.e., those with genital ulcers) are often missed by serological testing. The sensitivity of serological tests for primary syphilis is reported to be 62.5 %–78.4 % [2]. Primary syphilis can be diagnosed by detecting TP in a lesion, but there is no *in vitro* culture method for TP that can be implemented in clinical practice, and the sensitivity of dark-field microscopy examination is low. Nucleic acid amplification tests (NAATs) using ulcer/lesion swabs have been shown to be useful supplementary diagnostic tools for primary syphilis [3]. In addition, several studies have shown that high amounts of TP DNA can be detected in saliva, particularly at high frequencies in secondary syphilis (82.5 %–87.5 %) and latent syphilis (25.6 %–47.8 %) [4,5]. Saliva is expected to become an alternative diagnostic sample for patients who are reluctant to undergo blood sampling. The availability of NAATs as a point-of-care test (POCT) is expected to improve the diagnosis and treatment outcome for syphilis, and help to prevent further

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increases in the number of cases.

GeneSoC is a quantitative PCR (qPCR)-based diagnostic system (Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan) that utilizes microfluidic technology to perform rapid PCR, completing 50 PCR cycles within 15 min [6]. The GeneSoC platform is applied as a POCT for various infectious diseases, including severe acute respiratory syndrome coronavirus 2, influenza virus, and *Staphylococcus aureus* [6–8]. However, its utility for the diagnosis of syphilis has not been evaluated. This study aimed to assess the basic performance of the rapid qPCR assay using the GeneSoC platform for TP detection in clinical specimens.

In this study, 116 clinical samples of a suspension of an ulcer/lesion swab in phosphate-buffered saline (syphilis, $n = 43$; non-syphilis, $n = 20$) and saliva (syphilis, $n = 33$; non-syphilis, $n = 20$) collected from adult patients (≥18 years old) were used. These samples were collected and used in previous studies, and the diagnosis of syphilis was based on positive rapid plasma regain and TP latex agglutination serological tests [4,9]. The samples collected from patients with and without syphilis were confirmed as positive and negative by a NAAT, respectively, and were subsequently frozen for storage. qPCR for a single copy of the *polA* and *tp47* genes in TP has been used widely for the diagnosis of syphilis in clinical specimens. Previously, we confirmed that qPCR for *tp47* [10] has superior sensitivity compared with *polA* [11] in our in-house protocol [4]. Therefore, we chose conventional qPCR for *tp47* [10] as the reference method. Conventional qPCR was performed using TaqMan Fast Advanced Master Mix for qPCR (Applied Biosystems, Waltham, MA) on a QuantStudio 5 (Applied Biosystems). A positive result was determined according to a Ct value < 47 for *tp47* (FAM). Ten-fold serial dilutions of AmpliRun Treponema DNA Control (Vircell S.L., Granada, Spain) ranging from 2 to 2.0 × 10^3 copies/reaction were used to estimate TP DNA copy number in the specimens.

A rapid qPCR assay was performed using a GeneSoC *Treponema pallidum* Detection Kit for *polA* and a GeneSoC Mini 2. In brief, 12 μL reaction reagent was mixed with 6 μL primer/probe mix and 2 μL template, and the solution was dispensed onto a reaction panel chip for the GeneSoC Mini 2. The thermal cycling conditions were as specified in the manufacturer's instructions. All results were interpreted automatically by the GeneSoC Mini 2. A positive result was determined if the Ct value for *polA* (Cy5) was <50. A negative result was determined if the internal control had a Ct value < 50, but *polA* was not detected with a Ct value < 50. If neither the internal control nor *polA* was detected, the test was considered a failure and was repeated. The conventional and GeneSoC rapid qPCR assays were conducted in duplicate. If either of the two replicates was positive, the result was interpreted as positive.

Firstly, we evaluated the conventional and GeneSoC rapid qPCR assays using 10-fold serial dilutions of control TP DNA. The detection limit of the conventional and GeneSoC rapid qPCR assays was 2 copies/reaction within 45 cycles and 20 copies/reaction within 50 cycles, respectively (Fig. 1).

Subsequently, we evaluated the GeneSoC rapid qPCR assay using suspensions of ulcer/lesion swabs in phosphate-buffered saline. Purified DNA was extracted with a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), and TP DNA was quantified using conventional qPCR. TP DNA in 43 swab suspensions from patients with syphilis had a median of 1672 (interquartile range [IQR], 559–4502) copies/mL (Fig. 2). We confirmed that TP DNA was not detected in 20 swabs collected from

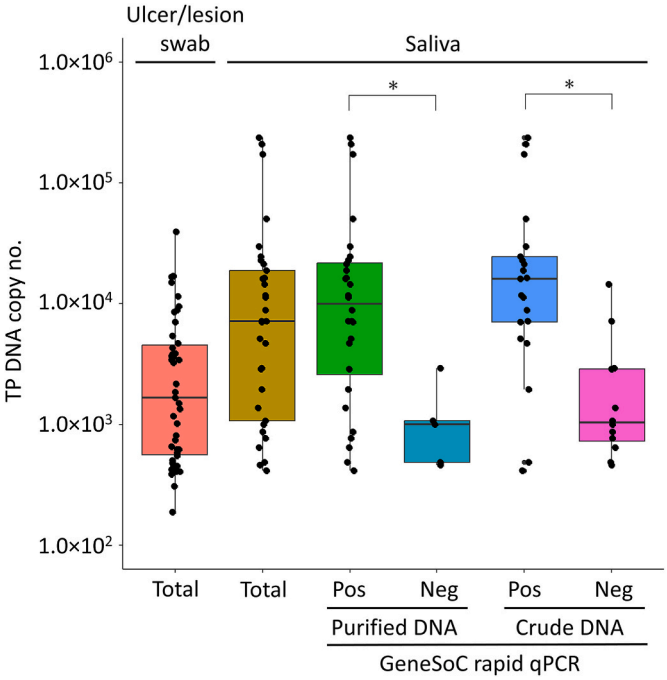

Fig. 2. TP DNA copy number in clinical samples. Ulcer/lesion swabs ($n = 43$) and saliva ($n = 33$) collected from patients with syphilis. Negative results for the GeneSoC rapid qPCR assay ($n = 5$ and $n = 12$ for purified DNA and crude DNA, respectively) were observed in clinical samples with a lower TP DNA copy number than in samples with positive results ($n = 28$ and $n = 21$ for purified DNA and crude DNA, respectively). Each dot shows TP DNA copy number in each sample. *$p < 0.05$, Mann–Whitney *U* test. Neg, negative; Pos, positive.

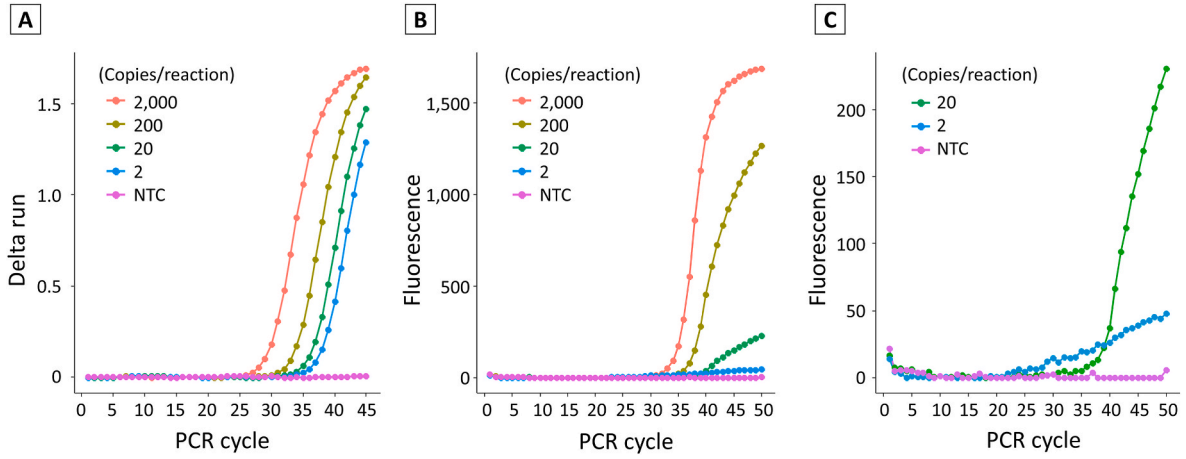

Fig. 1. Basic performance of the conventional and GeneSoC rapid qPCR assays. The limit of detection for the conventional qPCR assay for *tp47* on a QuantStudio 5 (A) and GeneSoC rapid qPCR assay for *polA* on a GeneSoC Mini 2 using a TP DNA dilution series from 2000 to 2 copies/μL, including a non-template control (NTC) (B), and results for the 20 to 2 copies/μL range and NTC (C).

patients without syphilis. For the GeneSoC rapid qPCR assay, we tested the direct method without DNA extraction (GeneSoC direct-rapid qPCR assay), according to the manufacturer's instructions; 2 μL of the suspension was added directly to the PCR mix, which was injected into a microchip, and the GeneSoC rapid qPCR assay was performed. The results of the GeneSoC direct-rapid qPCR assay with duplicate tests were fully consistent with those obtained from the conventional qPCR assay. For one specimen, a discrepancy was observed between the duplicate tests: one yielded a positive result, while the other was negative. The positive, negative, and overall agreement rate between both assays was 100 % (95 % confidence interval [CI], 91.8 %–100 %), 100 % (95 % CI, 83.2 %–100 %), and 100 % (95 % CI, 94.3 %–100 %), respectively (Table 1).

Furthermore, we evaluated the GeneSoC rapid qPCR assay using saliva samples. Of 33 saliva specimens that were positive by conventional qPCR with purified DNA, TP DNA in saliva samples from patients with syphilis had a median of 7148 (IQR, 1076–18,655) copies/mL (Fig. 2). TP DNA was not detected in 20 saliva samples collected from patients without syphilis. Due to air flow errors on the GeneSoC Mini 2 platform when injecting saliva directly into the microchip, we did not use the GeneSoC direct-rapid qPCR assay. Therefore, we performed crude DNA extraction as previously described [4] to simplify the testing method. In brief, saliva samples (50 μL) were centrifuged at 15,000×*g* for 3 min at room temperature, and the sediment was resuspended in 50 μL MightyPrep Reagent for DNA (Takara Bio, Inc., Kusatsu, Japan), and then heated at 95 °C for 10 min. The suspension was centrifuged at 15,000×*g* for 3 min at room temperature and the supernatant was used for further experiments. When crude DNA was used, the positive, negative, and overall agreement rate between the GeneSoC rapid and conventional qPCR assays was 63.6 % (95 % CI, 45.1 %–79.6 %), 100 % (95 % CI, 83.2 %–100 %), and 77.5 % (95 % CI, 63.8 %–87.7 %), respectively (Table 1).

Because the sensitivity was lower than expected, additional verification was performed using DNA purified from the saliva samples. The agreement rate was improved compared with crude DNA, with a positive, negative, and overall agreement rate of 84.8 % (95 % CI, 68.1 %–94.9 %), 100 % (95 % CI, 83.2 %–100 %), and 90.6 % (95 % CI, 79.3 %–96.9 %), respectively (Table 1). Disagreement between the GeneSoC rapid and conventional qPCR assays was observed in saliva samples with a low TP DNA copy number (median, 1003 [IQR, 482–1076] copies/mL for purified DNA, and median, 1039 [IQR, 730–2871] copies/mL for crude DNA) (Fig. 2).

This study is the first report to evaluate the basic performance of a rapid qPCR assay for syphilis in clinical samples using the GeneSoC platform. The detection limit of the GeneSoC rapid qPCR assay was 10 times higher than that of a conventional qPCR assay (TP DNA 20 copies/reaction vs. 2 copies/reaction, respectively), which was equivalent to that of a loop-mediated isothermal amplification assay (27.5 copies/reaction) [4]. For a NAAT to be utilized as a POCT, it is crucial to streamline the DNA extraction process and minimize the time required for PCR. In this study, we focused on the GeneSoC direct-rapid qPCR assay without DNA extraction. We found that the results of the GeneSoC direct-rapid qPCR assay were 100 % concordant with those of conventional qPCR using purified DNA. Because of its short sample preparation and reaction times, the turnaround time (TAT) of the GeneSoC assay was less than 40 min after sample collection. Considering its limit of detection and short TAT, the GeneSoC direct-rapid qPCR assay shows promise as a POCT for primary syphilis. By using the GeneSoC assay, the risk of missing treatment opportunities for patients with primary syphilis who might have a false negative result in serological testing is reduced. Additionally, its short TAT allows for an immediate diagnosis and treatment to be started on the same day. As of May 2025, the running cost per test is approximately 2000 JPY (~14 USD). While the assay involves some expense, it enables rapid diagnosis and immediate treatment, potentially eliminating the need for follow-up visits and additional consultation fees. Therefore, it is considered to offer substantial benefits to patients. It is important to note that the purpose of this study was to evaluate the concordance between the conventional and GeneSoC rapid qPCR assays using clinical samples. Our results do not reflect the actual diagnostic sensitivity and specificity in the clinical setting. The sensitivity of NAATs for ulcer/lesion swabs from patients with primary syphilis ranges from 72 % to 95 % [11,12]. Due to variations in patient lesions and sample collection techniques, there may be significant fluctuations in sensitivity, and attention should be paid to the possibility of false-negative results. In addition, when swab samples contain large amounts of blood or have high viscosity, the risk of false negatives or false positives may increase [13]. Further validation of the GeneSoC rapid qPCR assay using additional clinical samples is necessary, along with the development of appropriate sample collection and preparation techniques to address these challenges.

In this study, we evaluated the GeneSoC rapid qPCR assay using saliva samples as well as ulcer/lesion swabs, but the positive agreement rate with conventional qPCR was lower than expected (63.6 % for crude DNA and 84.8 % for purified DNA). Several reasons could explain why the GeneSoC rapid qPCR assay showed a lower positive rate compared with conventional qPCR. First, the detection limit of the GeneSoC rapid qPCR assay was higher than that of conventional qPCR, suggesting it lacked sufficient sensitivity for specimens with a low TP DNA copy number. Second, the impact of PCR inhibitors in saliva or crude DNA extracts could be a contributing factor. Even with purified DNA, not all PCR inhibitors may have been removed. Third, saliva samples contain a high amount of bacterial and human DNA [14], which could affect

Table 1
Agreement rate between the conventional and GeneSoC rapid qPCR assays.

		Conventional qPCR[a] with purified DNA[c]		Positive agreement rate % (95 % CI)	Negative agreement rate % (95 % CI)	Overall agreement rate % (95 % CI)
		Positive no.	Negative no.			
Ulcer/lesion swabs						
Direct-rapid qPCR[b]	**Positive no.**	43[e]	0	100 (91.8–100)	100 (83.2–100)	100 (94.3–100)
	Negative no.	0	20			
Saliva						
Rapid qPCR[b] with crude DNA[d]	**Positive no.**	21[f]	0	63.6 (45.1–79.6)	100 (83.2–100)	77.5 (63.8–87.7)
	Negative no.	12	20			
Rapid qPCR[b] with purified DNA[c]	**Positive no.**	28[g]	0	84.8 (68.1–94.9)	100 (83.2–100)	90.6 (79.3–96.9)
	Negative no.	5	20			

sensitivity. In the GeneSoC rapid qPCR assay, insufficient primer annealing to the target gene may have resulted in inadequate amplification. Although saliva is a highly valuable and easily collected sample, its use in the GeneSoC rapid qPCR assay will require the optimization and improvement of DNA extraction and reaction conditions.

In this study, qPCR results were interpreted as positive if either of the two replicates was positive. However, in clinical practice, testing is often performed with a single sample due to time or resource constraints. This may increase the risk of false-negative results, representing a limitation of the current approach. A further limitation is that the GeneSoC system for TP detection is currently only available in Japan. Therefore, its clinical benefits in other countries remain unverified. However, its strong performance and rapid TAT suggest the potential for its future global use.

In conclusion, the GeneSoC direct-rapid qPCR assay shows promise as a POCT for ulcer/lesion swabs in patients with primary syphilis. Further evaluations of this assay in prospective clinical trials are desired.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Review Committee at Saitama Medical University Hospital (approval number 2023–079). In this study, we used only the residual specimens from previous research [4,9]. Informed consent for secondary use was obtained by opt-out via the website.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

KI, study conceptualization; HM, RO, and KT investigation; AS, and MT, data collection and curation; HM, performing formal analysis; KI, HM, TM, manuscript drafting; YO, SN and YA, manuscript revision; TM, study supervision; YA, Funding acquisition. All authors have read and approved the final manuscript.

Competing interests

The authors report no conflict of interests relevant to the published work.

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