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Ultra-fast, sensitive and quantitative on-chip detection of group B streptococci in clinical samples

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ABSTRACT

PCR enables sensitive and specific detection of infectious disease agents, but application in point-of-care diagnostic testing remains scarce. A compact tool that runs PCR assays in less than a few minutes and that relies on mass-producible, disposable reactors could revolutionize while-you-wait molecular testing. We here exploit well-established semiconductor manufacturing processes to produce silicon ultra-fast quantitative PCR (UF-qPCR) chips that can run PCR protocols with limited assay optimization. A total of 110 clinical samples were analyzed for the detection of group B streptococci using both a validated benchtop and an on-chip qPCR assay. For the on-chip assay, the total reaction time was reduced after optimization to less than 5 min. The standard curve, spanning a concentration range of 5 log units, yielded a PCR efficiency of 94%. The sensitivity obtained was 96% (96/100; CI: 90–98%) and the specificity 70% (7/10; CI: 40–90%). We show that if melting analyses would be integrated, the obtained sensitivity would drop slightly to 93% (CI: 86–96%), while the specificity would increase to 100% (CI: 72% – 100%). In comparison to the benchtop reference qPCR assay performed on a LightCycler©96, the on-chip assay demonstrated a highly significant qualitative (Spearman's rank correlation) and quantitative (linear regression) correlation. Using a mass-producible qPCR chip and limited assay optimization, we were able to develop a validated qPCR protocol that can be carried out in less than five minutes. The analytical performance of the microchip-based UF-qPCR system was shown to match that of a benchtop assay. This is the first report to provide UF-qPCR validation using clinical samples. We demonstrate that qPCR-based while-you-wait testing is feasible without jeopardizing assay performance.

1. Introduction

The diagnosis of microbial infections traditionally relies on culture based techniques often followed by biochemical or antimicrobial susceptibility tests to direct clinical decisions [1]. More recently, nucleic acid test after enrichment may be used [2,3]. Especially for cases where rapid screening or treatment is needed, nucleic acid based tests have moved into the clinical laboratories for detection of agents causing infectious diseases, increasingly replacing classical culture-based microbiology techniques. Recent developments, which integrate sample preparation and PCR in a single automated workflow with limited hands-on time like Xpert® from Cepheid, go one step further, providing access to molecular testing at the point of care. Still, turnaround times of typically 1 h mean that true while-you-wait testing remains beyond reach. With techniques available that perform nucleic acid extraction in less than 10 min, ultra-fast (in under 5 min) quantitative PCR (UF-qPCR) is a target to effectively allow while-you-wait testing [4–6]. As noted previously [7], the present challenge for decreasing PCR amplification times lies with instrumentation, not with PCR chemistry. A substantial R&D effort is spent investigating technologies that can reduce reaction times, specifically by speeding up the thermal cycling that is fundamental to PCR. Examples include using an oscillating fluid

Abbreviations: UF-qPCR, ultra-fast qPCR; GBS, group B streptococci; NTC, no template control; Ct, cycle threshold

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plug [8], continuous flow-through [9–12], static reaction mixtures [13–15] or liquid-based thermalization [16,17], resulting in PCR times down to 15 s [5]. In these different reports, the described technologies have either not been characterized intensively for analytical performance [8,10,11,14–16], or sacrifice assay performance for speed [9,12,13,17], while none provide clinical data to support their usefulness in a healthcare setting. Most importantly, none of the solutions described to date offer the perspective of integration into a compact tool that relies on mass-producible, disposable PCR reactors.

In this article, we describe the development, application and validation of silicon UF-qPCR chips produced using industry-standard semiconductor device fabrication processes, allowing easy upscaling to robust mass production. We used the chips to run a previously validated PCR assay for the detection of *Streptococcus agalactiae* (Group B streptococci, GBS). This target was selected because a molecular bedside test for GBS, which is harmful for newborn health when transmitted from the mother during childbirth, would significantly improve current screening strategies during antenatal care. Current screening for GBS is most often performed during late pregnancy (i.e. at 35 weeks of gestation), and women found positive receive antibiotic prophylaxis when in labor. However, due to the specific dynamics of GBS colonization (GBS can appear or disappear within a day), and not due to the diagnostic tool used, many women receive antibiotics unnecessarily, when GBS was present at time of screening but no longer at delivery. Also, many women do not receive the necessary prophylaxis because GBS is present at the time of delivery, while it was not present at the time of screening. Therefore, a true improvement would be a diagnostic tool that allows screening when women come in the hospital for delivery [18]. GBS detection therefore makes an excellent case for point-of-care DNA-based diagnostics and motivated the transfer of the assay to a fast chip format. We successfully analyzed 110 clinical samples on chip. After limited assay optimization, we obtained a high sensitivity and specificity confirming the strong agreement between the benchtop reference qPCR assay and the chip-based UF-qPCR assay performed in under 5 min per sample.

2. Materials and methods

2.1. Clinical Samples

Samples were obtained in a previous study [2]. One-hundred women at 35–37 weeks of gestation, attending the prenatal clinic at Ghent University Hospital, Ghent, Belgium (with an average of 1200 deliveries per year), were enrolled in the study from June 2009 to January 2010. The study was approved by the Ethics Committee (IRB protocol nr. 2007/096) of the Ghent University Hospital, Ghent, Belgium. All women provided informed consent prior to collection of samples.

Rectovaginal, vaginal and rectal samples were collected using nylon flocked swabs that were submerged into 1 mL of ESwab transport medium (ESwab, Copan Diagnostics, Brescia, Italy). Volumes of 200 µL from the ESwab transport medium of the rectovaginal ESwabs were inoculated into separate tubes with 5 mL of Todd-Hewitt broth with 1% yeast extract, 15 µg/mL nalidixic acid and 10 µg colistin/mL (Lim broth, Becton Dickinson, Erembodegem, Belgium). The tubes were incubated aerobically overnight at 37 °C. DNA was extracted as described previously [2] and stored at − 20 °C until use.

2.2. Silicon chip fabrication

The silicon chip (see Fig. 1A) was fabricated as described by Majeed and colleagues [19]. In summary, the processing of chips is based on etching the fluidic structures in silicon, sealing them by anodic bonding with a Pyrex wafer and opening access holes from the silicon backside. The qPCR reaction chambers on the silicon chip have a nominal depth of 250 µm, a width of 500 µm and a serpentine-shape design to overcome air trapping (Fig. 1B) resulting in a total reactor volume of 2.4 µL. Chips had multiple PCR cavities depending upon design (Fig. 1A), but only a single cavity was used for each PCR tests. Insulating trenches were etched around the reaction chamber (Fig. 1A) to avoid undesirable heating of the bulk of the chip during thermal cycling.

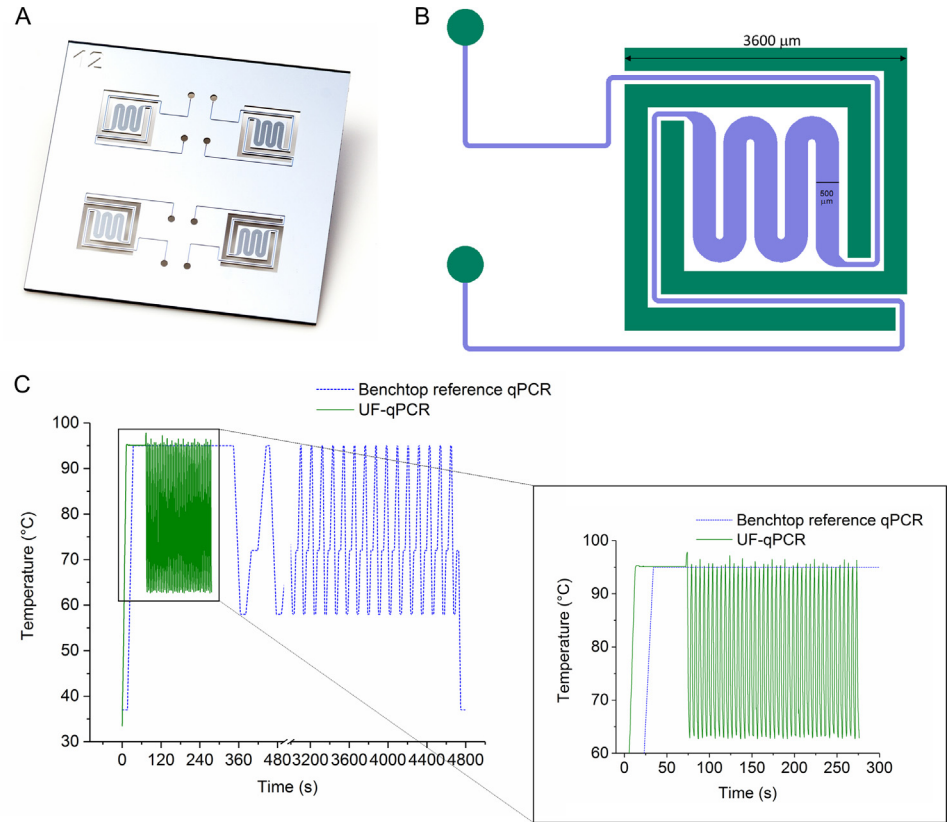

Fig. 1. A silicon-based chip was used to perform UF-qPCR. A) Photograph of the silicon chip with four reaction chambers. Each reaction chamber is enclosed by insulating trenches. B) Mask design of the silicon chip with an insulated reactor width of 3600 µm. C) Temperature versus time trace of benchtop reference qPCR assay versus UF-qPCR. The inset shows that the UF-qPCR assay is completed before initial denaturation has ended on the benchtop tool.

2.3. Experimental setup

The silicon chip was mounted with a thermo-electric cooler (#MPC701, Micropelt, Umkirch, Germany) for heating and cooling. A K-type thermocouple (#CHAL-010, Omega, Norwalk, USA), fabricated from a 25 µm diameter wire, was glued to a heat spreader for temperature monitoring (Fig. S1A). The thermo-electric cooler and heat spreader were stacked and glued together to a heat sink (Fig. S1A and S1B). A closed-loop PID controller with H-bridge switch (FTC200, Accuthermo Technology, Fremont, USA) was used to configure and to control the thermal cycling of the silicon chip. The Pyrex bonded to the silicon chip allows real-time fluorescence monitoring of the reaction chamber during thermal cycling using an inverted microscope (IX71, Olympus, Tokio, Japan) equipped with a CMOS camera (Orca Flash 4.0, Hamamatsu, Hamamatsu city, Japan) and fluorescent light source (X-Cite exacte, Excelitas Technologies, Waltham, USA).

2.4. PCR protocols

The *S. agalactiae sip* gene encoding the surface immunogenic protein was chosen as a qPCR target, generating a 78-bp long fragment, as described before [2].

2.5. Benchtop reference qPCR assay (performed with hydrolysis probe)

The benchtop reference qPCR assay was performed as before with minor modifications [2]. In short, we used the LightCycler© Probes Master kit (Roche, Basel, Switzerland) combined with *sip* primers (500 nM each) and *sip* probe (200 nM) and ran the assay on the LightCycler©96 (Roche, Basel, Switzerland) in a total volume of 10 µL. Cycling conditions for the LightCycler©96 were: 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 58 °C for 15 s and 72 °C for 20 s. The total run time including ramp rates was 1 h 20 min.

2.6. Standard on-chip qPCR assay (performed with an intercalating dye)

For the standard on-chip qPCR assay, a master mix with a total volume of 2.4 µL was prepared containing 1x KAPA2G M master mix (Kapa Biosystems, Wilmington, USA), 250 nM of *sip* primers, 2 mM of dNTPs (Invitrogen, Carlsbad, USA), 1x EvaGreen (Biotium, Fremont, USA), 2 mM of $MgCl_2$ (Invitrogen Carlsbad, USA), 1% sulfolane (Sigma, Missouri, USA), 0.5 mg/mL ultrapure bovine serum albumin (Ambion, Foster city, USA), and 0.02 units/µL of the 4:1 mix of KAPA2G fast polymerase and KAPA2G hot-start fast polymerase (Kapa Biosystems, Wilmington, USA). Following cycling conditions were used: 1 min initial denaturation followed by 40 cycles of 98 °C for 1 s and 63 °C for 15 s. The total run time including ramp rates was 17 min.

2.7. On-chip UF-qPCR assay (performed with an intercalating dye)

For the UF-qPCR assay, the same master mix was used as described before, but with increased primers and polymerase concentration: 20 µM of *sip* primers and 1.68 units/µL of KAPA2G HS fast polymerase (Kapa Biosystems, Wilmington, USA). Cycling conditions for UF-qPCR were 1 min initial denaturation at 95 °C, followed by 40 cycles of 98 °C for 1 s and 63 °C for 2 s. The total run time including ramp rates is 4 min 36 s.

The qPCR mixtures were pipetted into the reaction chamber of the chip for both on-chip methods.

2.8. Reference Sample

Standard dilution series were prepared using freeze-dried DNA from *S. agalactiae* strain 2603 (BAA-611D-5, ATCC, Manassas, USA), which was purchased and resuspended in DEPC-treated water (Invitrogen, Carlsbad, USA). The genomic concentration (genome copies/mL) of the DNA was calculated using the measured DNA concentration (Nanodrop 2000, Thermo Scientific, Waltham, USA), the genome size and GC% of the strain [20].

To obtain the same genome copy number per reaction, we took into account the difference in reaction volume between the benchtop reference qPCR assay and the on-chip assays. The latter one has a 4.17 times smaller reaction volume. Hence, for the calibration curves ranging from 10^5 down to 10^1 genome copies/reaction, we used 10-fold serial dilutions from 5×10^7 down to 5×10^3 genome copies/mL for the benchtop reference qPCR assay and 10-fold serial dilutions from 2.08×10^8 down to 2.08×10^4 genome copies/mL for the on-chip assays.

To determine the limit of detection (LOD), a concentration of 6.24×10^3 genome copies/mL of GBS DNA was tested 20 times in the UF-qPCR assay to assess the 95% probability of detecting 3 genome copies/reaction as defined by the MIQE guidelines [21]. Results were used to determine the LOD prediction interval.

2.9. Melting protocol

After on-chip qPCR, the reaction mixtures were removed from the chip by aspiration, and melting curves were obtained using a LightCycler©96 instrument (Roche, Base, Switzerland). Following protocol was used: 95 °C for 10 s, 65 °C for 60 s and then a continuous temperature increase, with a ramping rate of 0.2 °C/s until 97 °C. Melting peaks were calculated with the LightCycler©96 high resolution melting software.

2.10. Image acquisition

For the standard on-chip qPCR assay, a custom LabView script with automated filter wheel control was used to steer temperature cycling and to automate the image acquisition of the reaction chamber every cycle.

For the UF-qPCR assay, a movie was recorded instead. Using the HCImage software (Hamamatsu, Hamamatsu city, Japan) the movie was decomposed into individual images at a rate of 4 frames per second, resulting in over 1100 images per on-chip qPCR reaction. The image with the highest mean grayscale value relative to the previous and subsequent image (i.e. acquired at 63 °C) was used to build the amplification curve.

2.11. Data analysis

For the benchtop reference qPCR assay, raw data from the LightCycler©96 was used to determine the Ct value by the crossing point method Cy0 in R [22] from the qpcR package [23].

For the on-chip assays, image analysis (using ImageJ software (NIH, Bethesda, USA)) and the Cy0 method were used to determine the Ct value. More details are available in the Supplemental methods.

2.12. Statistical analysis

Concentrations were analyzed using the JMP software (SAS Institute, Cary, USA). Normality was tested using the Shapiro-Wilk test. Statistical hypothesis tests (regression, *t*-tests and equivalence tests) were considered significant for p values < 0.05.

Correlation of the benchtop reference qPCR and UF-qPCR results was determined using Spearman's rho value (ρ). The Bland-Altman method was used to visualize agreement between the two methods. Kappa values (κ) were used to assess the proportion of agreement between both methods for assigning a positive or negative result.

3. Results

We selected a previously validated, hydrolysis probe-based qPCR

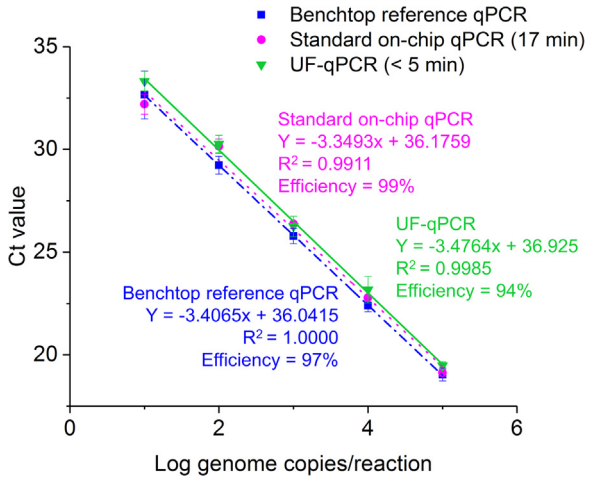

Fig. 2. Calibration curves for the GBS qPCR performed with the benchtop reference qPCR assay (LightCycler©96) and with two on-chip assays. The benchtop reference qPCR assay (N = 7) represented by blue squares and the fitted line shown as a blue dashed-and-dotted line, standard on-chip qPCR assay of 17 min (N = 2) represented in fuchsia and the fitted line shown as a fuchsia dotted line and UF-qPCR assay of 4 min 36 s (N ≥ 4) represented in green triangles with the fitted line shown as a green full line, with efficiencies of 97%, 99% and 94% respectively.

assay for the quantification of *S. agalactiae* (group B streptococci, GBS) in clinical samples and used it with minor modifications [2]. As the benchtop reference method, this assay was run on a standard, commercial qPCR tool (LightCycler©96). Assay performance was verified by analyzing a 10-fold dilution series of an *S. agalactiae* reference sample with known number of genome copies to establish a standard curve, showing an efficiency of 97% (Fig. 2). Initial transfer of the assay from benchtop to microchip was performed with little adaptation of master mix composition (as defined by the manufacturer) and of cycling conditions, as an intermediate step in the process of speeding up the PCR. We refer to this method as our standard on-chip qPCR assay. The cycling time was decreased from 163.4 s/cycle to 23.7 s/cycle, resulting in a total reaction time of 17 min with a PCR efficiency of 99% (Fig. 2). To increase the reaction speed of the standard on-chip qPCR assay further, we increased primers and KAPA2G polymerase concentration by 4 and 10 times, respectively. Increasing the concentration of the KAPA2G polymerase mix (with and without hot-start) generated primer-dimers in the no template control (NTC). Increasing the KAPA2G hot-start polymerase and omitting the KAPA2G without hot-start polymerase in the qPCR reaction enabled us to decrease the assay time from an initial 163.4 s/cycle (benchtop assay) to 4.8 s/cycle (on-chip) as shown in Fig. 1C. Including initial denaturation, we could perform qPCR within 4 min 36 s (see Supplemental video). The UF-qPCR on chip was completed before initial denaturation had finished on the benchtop tool (Fig. 1C).

Supplementary material related to this article can be found online at doi:10.1016/j.talanta.2018.09.041.

A calibration curve was used to determine the efficiency of the speeded up UF-qPCR assay as described for the standard on-chip qPCR. Fig. 2 shows an efficiency of 94%. Amplification curves are shown in Fig. 3A. To verify the specificity of the amplification of the UF-qPCR, we performed melting curve analysis. Fig. 3B shows clear melting peaks with the expected value (75 °C ± 0.42 °C) for each concentration. The limit of detection (LOD) for the UF-qPCR assay was determined. The theoretically lowest copy number that can be detected with 95% probability according to Poisson statistics is 3 copies per reaction, which corresponds to a concentration of 6.24×10^3 genome copies/mL (Ct = 34.60). The UF-qPCR assay reaches this theoretical limit as 19 experiments out of 20 did showed amplification. The 95% prediction interval for the LOD ranges from 1.70×10^4 to 1.01×10^3 genome copies/mL, corresponding to Ct values from 33.12 to 37.23.

To assess clinical performance of the UF-qPCR assay, we quantified GBS in DNA extracted from 110 patient samples, comprising 27 vaginal, 28 rectal, 27 vaginorectal swabs and 28 LIM broth-enriched samples. The latter enriched samples were quantified by the chip PCR as containing between 6log5 and 4.7log10 CFU GBS/mL. For sensitivity, samples with Ct values below 37.23, were considered positive. For specificity, samples that showed no amplification (i.e. Ct values above 37.23) were identified as negative. The benchtop reference qPCR identified 100 out of 110 samples as positive. UF-qPCR detected GBS in 96 of the 100 positive samples, which corresponds to a 96% (CI: 90–98%) sensitivity. Seven out of 10 samples were correctly identified as negative by UF-qPCR, which corresponds to a specificity of 70% (CI: 40–90%). Melting analysis, which was afterwards carried out off-chip, indicated the absence of a specific melting peak for all those 10 samples. Hence, if melting curve analysis were to be included on-chip, all true-negative samples would have been correctly identified, resulting in a specificity increase to 10/10 or 100% (CI: 72–100%). At the same time, however, an incorrect melting peak was observed for 3 out of the 96 positive samples, which would lead to a decrease in sensitivity from

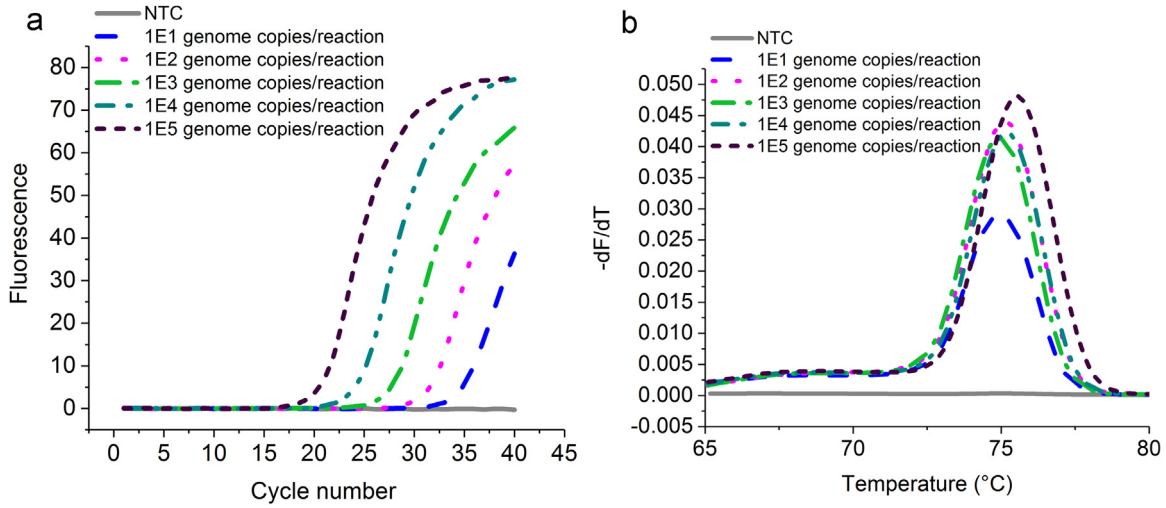

Fig. 3. Amplification (A) and melting curves (B) for a 10-fold dilution series of a reference sample of *S. agalactiae* genomic DNA, with concentrations spanning 5 log units and the no template control (NTC) performed with the UF-qPCR assay. A) Fluorescence versus cycle number. B) First derivative plots of the melting curves showing the specific melting peaks.

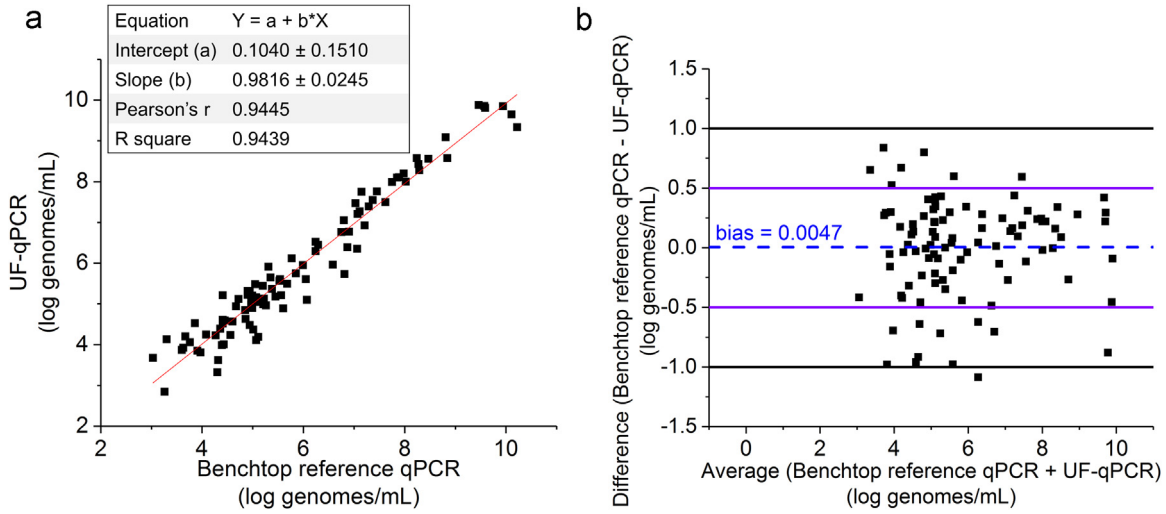

Fig. 4. Comparison of quantification results for GBS in the clinical samples as obtained using benchtop reference qPCR assay (X-axis) versus UF-qPCR (Y-axis). Statistical analysis of the patient samples using A) regression analysis, which shows that the correlation between UF-qPCR and benchtop reference qPCR assay quantification results is highly significant and B) Bland-Altman plot, which shows strong agreement between the UF-qPCR and benchtop reference qPCR assay.

96% (96/100) to 93% (93/100 and CI: 86–96%).

We investigated the agreement between the UF-qPCR and the benchtop reference qPCR quantification results in various ways. First, correlation between UF-qPCR and the benchtop reference qPCR quantification results is highly significant as indicated by Spearman's $\rho = 0.9445$, $p < 0.001$. Second, as shown in Fig. 4A, a simple linear regression between the two kinds of quantification results produced an intercept ($p = 0.6066$) and a slope ($p = 0.4553$) that are not significantly different from 0 and 1, respectively. Third, we conducted an equivalence test for the two kinds of quantification results [24]: the two one-sided *t*-test procedure shows that the calculated 95% confidence interval of the mean difference between the two tests (−0.1456 to 0.1590) falls within the equivalence region (−0.5 to 0.5). Fourth, we assessed the agreement between the two assays using a Bland-Altman plot. The Y-axis in that plot shows the log difference of the UF-qPCR assay and benchtop reference qPCR assay, while the X-axis shows the average log of the two assays (Fig. 4B). The bias between the two assays is 0.0047, whereas 81% and 99% of the results lie between − 0.5 and 0.5 log genome copies/mL and − 1.0 and 1.0 log genome copies/mL [25], respectively, indicating strong agreement between the two assays.

When performing a qualitative analysis of the UF-qPCR assay and the benchtop assay by only looking at positive and negative observations, the Kappa coefficient κ indicates an agreement between the UF-qPCR and benchtop reference qPCR assay results of 0.74 with and 0.63 without melting curve analysis. The significance for both these values is $p < 0.0001$.

4. Discussion

The screening for GBS during pregnancy is part of a routine check-up performed at 35–37 weeks of gestation. GBS present in rectum and/or vagina can be transmitted to the newborn during childbirth, potentially leading to pneumonia, meningitis and/or death of the neonate. This screening strategy is aimed at the identification of the 10–30% of women who are vaginal and/or rectal GBS carriers who should receive intrapartum antibiotic prophylaxis. This antepartum test for GBS does not accurately predict genital tract colonization at the time of labor [26]. As a consequence, women found negative at screening but positive at labor will be withheld treatment and, vice versa, women who convert from positive to negative will receive unnecessary treatment. Furthermore, women who deliver preterm or enter late into prenatal care do not get the chance to be screened at 35–37 weeks of gestation, and therefore, their GBS status is not known at the time of labor [3,26–28]. This indicates the need for a rapid and sensitive test that allows intrapartum detection of GBS. Hence, the UF-qPCR-based test described here could be the catalyst for the development of a point-of-care assay, not only to limit the number of false-negative test results, but also to decrease the overuse of antibiotics. A regular qPCR assay takes commonly at least one hour whereas the on-chip UF-qPCR assay described here is completed within 4 min 36 s. The speed improvement was only possible after increasing polymerase and primers concentration. For the polymerase, we used a hot-start polymerase instead of a combined mixture of non-hot-start and hot-start polymerases to avoid primer-dimer formation. Also, an intercalating dye instead of a Taqman probe was used, due to the fact that at present the fastest polymerases on the market do not have 5′ exonuclease activity [29]. Importantly, the decrease in runtime did not affect the efficiency of the qPCR assay.

Statistical analysis by means of Spearman's ρ, and linear regression show that there is strong correspondence between the results obtained using the on-chip UF-qPCR assay and the benchtop reference qPCR (Fig. 4A). In addition, the two one-sided *t*-test indicates statistical equivalence of the two assays and a Bland-Altman plot (Fig. 4B) shows that the quantification is in good agreement [25]. Finally, for the qualitative comparison, the Kappa coefficient κ also indicates a strong agreement by far exceeding any level of agreement that can be explained by chance alone [30].

Use of melting curve analysis after UF-qPCR increased assay specificity, but decreased assay sensitivity. Three samples were identified correctly as positive, i.e. in agreement with the benchtop reference qPCR method, but these samples did not show a correct melting peak after UF-qPCR and should therefore be considered negative when taking into account melting curve data. For two of these samples, concentrations were indeed lower than the theoretical LOD of the UF-qPCR assay due to the fact that the chip reaction volume is 4.17 times smaller than that of the benchtop reference qPCR assay. This can only be improved by increasing reaction cavity volume and, hence, chip size. On the other hand, specificity increased from 70% to 100% by including melting analysis. The three samples that were false-positive could be corrected and considered as negative by taking the melting curve data into account. Although adding melting curve analysis to the UF-qPCR will evidently increase the run time of the assay, recent work by Pryor and colleagues indicates that it is possible to perform high resolution melting within 1 s [31], suggesting that the increase is negligible.

As mentioned above, the cavity volume of the UF-qPCR described here is more than four times smaller than that of the benchtop reference

qPCR assay, which is an advantage for sample consumption. The reagent cost was, however, not reduced as we needed to increase the amount of polymerase to speed up the qPCR reaction. We only tested three different polymerase concentrations, it could be useful to do a more thorough screening of the amount of polymerase to be used. The chips used during the experiments were also relatively large and contained multiple cavities. In the future, we envision the use of smaller, single-cavity, single-use chips as the chip cost scales with its size.

The development of UF-qPCR, possible by combining well-established semiconductor chip technology and molecular biology tools, can speed up the commercialization of fast commercial qPCR tools, which are currently slowly entering the market, for example the *xxpress* from BJS Biotechnologies (40-cycle qPCR in 10 min: Xxpress from BJS Biotechnologies) and the *genechecker* from Genesystem (30-cycle qPCR in 12 min: Genechecker from Genesystem Co., Ltd). Ultimately, however, the goal is to combine fast qPCR with integrated sample preparation, realizing while-you-wait testing in under 15 min. By combining techniques that perform nucleic acid extraction in under 10 min [32], and UF-qPCR chips that perform amplification in under 5 min, we are close to reaching this goal. The fastest currently available commercial solution to offer a DNA-based sample-to-answer result for GBS testing is the GeneXpert from Cepheid, which requires 50 min (Xpert® GBS from Cepheid). UF-qPCR could also outperform antibody-based approaches such as lateral flow immunoassays, both in terms of speed and sensitivity [33,34].

In conclusion, our research shows that it is possible to accurately and efficiently quantify nucleic acids using qPCR within 5 min. We expect that combining fast DNA extraction, UF-qPCR and fast high-resolution melting, producing a complete sample-to-result device that requires less than 15 min from start to finish, may soon be feasible. We hope that our work will contribute to the development of these fast and accurate diagnostic devices for use at the point of care.

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Conflict of interest

We declare that we have no conflict of interest.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2018.09.041.

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