Evaluating preanalytical sample storage parameters for nucleic acid-based detection of *Neisseria gonorrhoeae*

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**ABSTRACT**

**Objectives** To ensure accurate diagnosis of infectious diseases, preanalytical factors should be considered when assessing specimen quality and subsequent test result. Accordingly, we aimed to systematically assess the effect of storage time, temperature and buffer on the analytical sensitivity of detecting the sexually transmitted pathogen, *Neisseria gonorrhoeae* across multiple molecular diagnostic platforms.

**Methods** Cultured *N. gonorrhoeae* was spiked into generic and commercial storage buffers and stored at four temperatures and five time points, ranging from −20°C to 37°C, over 30 days. Samples were processed using the Alinity m STI, Xpert CT/NG and Aptima Combo 2 nucleic acid amplification assays and an in-house quantitative PCR assay. A reduction in analytical sensitivity was defined as a significant (p<0.05) increase in cycle threshold (Ct) value relative to control samples.

**Results** In total, 2756 samples were processed, with *N. gonorrhoeae* detected in 99.2% of samples. With respect to time, analytical sensitivity was maintained from day 2 (113/120; 94.2%) up to day 30 (110/120; 91.7%) relative to baseline samples. With respect to temperature, analytical sensitivity was maintained from −20°C (147/150; 98.0%) up to 37°C (136/150; 90.7%) relative to baseline samples. Generic buffers, Viral Transport Medium and Amies Liquid Media showed a reduction in analytical sensitivity compared with their commercial counterparts, Aptima Multitest Swab Transport Media and Abbott Alinity transport buffer using select diagnostic assays; this reduction appeared temperature dependent, with the largest differences in median Ct values observed at 37°C (p<0.05).

**Conclusions** Increased prevalence of sample self-collection for sexually transmitted infections (STIs) warrants an evaluation of preanalytical sample storage variables on diagnostic testing performance. Here, across a range of time points, temperatures and storage buffers, *N. gonorrhoeae* was successfully detected, supporting flexibility in sample storage, and by extension the feasibility of analysing self-collected samples to improve access to STI testing.

**WHAT IS ALREADY KNOWN ON THIS TOPIC**

⇒ Despite the increased prevalence of sample self-collection for sexually transmitted infections (STIs), comprehensive evaluation of pre-analytical sample storage variables on diagnostic testing performance has not been conducted.

**WHAT THIS STUDY ADDS**

⇒ Previous studies have shown that sample integrity is best maintained under −4°C. Here we extended this knowledge by investigating the effects of temperature, time and storage buffer on the detection of *N. gonorrhoeae*.

⇒ Storing *N. gonorrhoeae* samples for up to 30 days at −20°C to 37°C prior to testing, did not impact analytical sensitivity on an array of diagnostic platforms. While modest storage buffer specific reductions in analytical sensitivity were observed.

**HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY**

⇒ Overall findings support flexibility in sample storage, and by extension the feasibility of analysing self-collected samples to improve access to STI testing.

INTRODUCTION

Globally, over 1 million sexually transmitted infections (STIs) are acquired daily, including gonorrhoea, which in 2020 caused 82.4 million new cases.¹ Considered a curable STI, gonorrhoea is transmitted by the gram-negative, diplococcus, human obligate bacterium *Neisseria gonorrhoeae*.
analytical sensitivity of *N. gonorrhoeae* detection across molecular diagnostic platforms.

**METHODS**

**Study design and sample preparation**

The well-characterised, laboratory *N. gonorrhoeae* FA1090 strain was cultured for 48 hours at 37°C on chocolate agar in triplicate. To prepare test samples with defined starting concentrations, suspensions of cultured *N. gonorrhoeae* were prepared at 3 McFarland standards using the VITEK DensiChek (BioMérieux, Marcy-l’Étoile, France) instrument according to manufacturer’s instructions, with phosphate-buffered saline used for resuspension. Suspensions were then pelleted by centrifugation (16 000×g for 2 min), the supernatant removed and pellets resuspended in 1 mL of storage buffer; two generic (Amies Liquid Media and Viral Transport Medium (VTM)) and two commercial (Aptima Multitest Swab Transport Media and Abbott Alinity transport buffer). Storage buffers were selected based on those commonly used in our jurisdiction, varying lytic properties, compositions and price points. Importantly, Amies Liquid Media was included in this study as it allows for *N. gonorrhoeae* culture. Each sample was serially diluted through the addition of further storage buffer to *N. gonorrhoeae* colony-forming unit estimated concentrations (CFU/mL) of 51 840 CFU/mL (high), 518.4 CFU/mL (middle) and 51.84 CFU/mL (low). Samples of each concentration were aliquoted into tubes representing day 0 at 25°C (samples processed on study commencement), or 1 of 20 pairwise sets based on five time points (2 days, 4 days, 7 days, 14 days and 30 days) and four temperatures (−20°C, 4°C, 25°C and 37°C) (figure 1).

**Sample processing**

Samples were processed for *N. gonorrhoeae* detection using the Alinity m STI assay (Abbott, Chicago, Illinois USA) on the Alinity m System, Aptima Combo 2 assay (Hologic, Marlborough, Massachusetts, USA) on the Panther System and Xpert CT/NG assay (Cepheid, Sunnyvale, California, USA) on the GeneXpert System (figure 1); each in accordance with manufacturer’s recommendations. Additionally, genomic DNA (gDNA) extracted using the EZ1&2 Virus Mini Kit v2.0 (QIAGEN, Hilden, Germany) on the EZ1 Advanced XL platform was used for an in-house quantitative PCR (qPCR) assay performed on the QuantStudio 5 (ThermoFisher Scientific, Waltham, Massachusetts, USA) targeting the *N. gonorrhoeae* specific, single-copy porA pseudogene (figure 1; online supplemental table S1).³

**Data analysis**

Prism V9.4.0 was used for all statistical analyses performed. Baseline samples were processed on day 0 at 25°C. Two-way repeated measures analysis of variance (ANOVA) (Šidák correction model) was used to determine the effect of time and temperature, with adjusted p values used to account for the interaction between experimental parameters. In this study using a laboratory strain under controlled conditions, a reduction in microbial load detection is defined by a significant increase in cycle threshold (Ct) value relative to baseline samples (p value <0.05) and referred to as a reduction in analytical sensitivity. This differs from diagnostic sensitivity which typically refers to the ability to detect a pathogen, presence/absence from clinical samples.⁶ Unpaired t-tests were performed to compare median readout values for analytical sensitivity between storage buffers (p value <0.05) and substantiated using 95% CIs of the median. Invalid and/or
Effect of storage buffer on analytical sensitivity of *N. gonorrhoeae* detection

All buffers were interrogated using the Alinity m STI assay. A significant reduction (p<0.05) in analytical sensitivity was observed for samples stored in Amies Liquid Media with the greatest reduction in analytical sensitivity observed for low concentration samples (median Ct value 36.8; 95% CI 36.5 to 37.6, n=59) compared with all other buffers: Aptima Multitest Swab Transport Media (median Ct value 31.5; 95% CI 31.0 to 31.8, n=61), Abbott Alinity transport buffer (median Ct value 32.4; 95% CI 32.1 to 32.8, n=63) and VTM (median Ct value 32.0; 95% CI 31.6 to 32.7, n=61). Regardless of concentration, differences in analytical sensitivity between buffers appeared to be associated with temperature, with variations most evident at 37°C (figure 2; online supplemental figures S1D, S2D and S3D). Low concentration samples stored in generic buffers, Amies Liquid Media (median Ct value 37.1; 95% CI 36.5 to 39.0, n=15) and VTM (median Ct value 34.8; 95% CI 33.2 to 36.8, n=15) had significantly higher median Ct values (p<0.05) compared with the commercial buffers, Aptima Multitest Swab Transport Media (median Ct value 31.5; 95% CI 30.1 to 32.1, n=15) and Abbott Alinity transport buffer (median Ct value 31.8; 95% CI 31.5 to 33.5, n=15).

VTM and Amies Liquid Media samples were also processed using the Xpert CT/NG assay (NG2 target), with a temperature-dependent difference in buffer performance again observed at 37°C, irrespective of concentration (figure 2; online supplemental figure S4D, S5D and S6D); low concentration samples stored in VTM (median Ct value 31.3; 95% CI 30.7 to 33.0, n=17) had a significantly (p<0.05) higher median Ct value compared with Amies Liquid Media (median Ct value 28.7; 95% CI 28.3 to 29.1, n=18).

All buffers were evaluated using an in-house qPCR assay for the *porA* target gene. Consistent with the other assays, a temperature-dependent difference in buffer performance was observed at 37°C for high and middle concentration samples (online supplemental figures S7D and S8D); high concentration samples stored in VTM (median Ct value 28.9; 95% CI 28.1 to 29.8, n=15) had a significantly higher (p<0.05) median Ct value compared with Aptima Multitest Swab Transport Media (median Ct value 26.2; 95% CI 25.8 to 26.8, n=15), Abbott Alinity transport buffer (median Ct value 26.3; 95% CI 26.0 to 27.4, n=15) and Amies Liquid Media (median Ct value 25.7; 95% CI 25.1 to 26.3, n=15) (online supplemental figure S7D). However, contrary to the other assays performed, significant differences between buffers were not observed at 37°C for low concentration samples processed using qPCR for the *porA* target gene (online supplemental figure S9D); most likely attributable to several VTM samples (8/15; 53.3%) exceeding qPCR limit of detection under these testing parameters. Overall, these results demonstrate that while subtle reductions in analytical sensitivity were observed for select buffers, there is certainly scope for context-specific flexibility in storage buffer selection.

DISCUSSION

To improve access to diagnostic services, the self-collection of patient samples has become increasingly prevalent, offering a wide-reaching and novel framework for gonorrhoea diagnosis. However, limited emphasis has been placed on identifying the acceptable limits of parameters required for maintaining *N. gonorrhoeae* sample integrity during sample collection, storage and transport to ensure accurate downstream test performance. Here, our results extend on current literature and highlight failed tests due to instrumental or sample processing errors were excluded from data analyses.

RESULTS

Summary of *N. gonorrhoeae* detection across processed samples

*N. gonorrhoeae* was detected in the majority of successfully processed samples (2734/2756; 99.2%). Seventy-nine processed samples encountered instrumental error—Aptima Combo 2 assay (40/79; 50.6%), Alinity m STI assay (22/79; 27.8%), Xpert CT/NG assay (17/79; 21.5%) and qPCR assay (0/79; 0%). Due to the high rate of instrumental errors observed using the Aptima Combo 2 assay and because the assay readout is relative light units (×1000) rather than Ct values, data collected on this platform was not used for subsequent analysis (online supplemental tables S2A–C).

Effect of time and temperature on analytical sensitivity of *N. gonorrhoeae* detection

With respect to time, analytical sensitivity was maintained across the range of time points tested, at day 2 (113/120; 94.2%), day 4 (108/120; 90.0%), day 7 (110/120; 91.7%), day 14 (105/120; 87.5%) and day 30 (110/120; 91.7%), irrespective of the other experimental variables and molecular assay performed (figure 2; online supplemental figures S1–S9). With respect to temperature, analytical sensitivity was highest for samples stored at −20°C (147/150; 98.0%) and 4°C (147/150, 98.0%). However, a relatively high analytical sensitivity was also observed for samples stored at 25°C (139/150; 92.7%) and 37°C (136/150; 90.7%) (figure 2; online supplemental figure S1–S9); reductions in analytical sensitivity observed at these temperatures are likely interrelated to the other variables tested. Overall, these results demonstrate that in a controlled laboratory setting, flexibility in sample storage can be afforded for up to 30 days and 37°C.

![Figure 2](Image 79x545 to 252x746)

The effect of time (at 37°C) and temperature (at 30 days) on *Neisseria gonorrhoeae* analytical sensitivity. Represented samples of low starting *N. gonorrhoeae* concentration were stored in Viral Transport Medium (VTM), Amies Liquid Media (Amies), Aptima Multitest Swab Transport Media (Aptima) or Abbott Alinity transport buffer (Alinity). Ct, cycle threshold; qPCR, quantitative PCR.
that service providers can likely deviate from stringent sample storage standards to ensure continuity in service delivery; particularly important during consumable shortages and for outbreak preparedness.\textsuperscript{3, 4} In saying that, the reduced performance of generic storage buffers observed in this study may compromise downstream bacterial culture and/or next-generation sequencing, for which sufficient microbial load is required for a fully informative analysis\textsuperscript{7}; although this was not tested in our study. In addition, our findings suggest that VTM should not be used for samples processed using the Xpert CT/NG assay. Importantly, our findings were applicable across a range of routinely used diagnostic platforms; however, due to instrumental errors caused by the Panther System, trends attributable to the effects of time and temperature were unable to be discerned from the Aptima Combo 2 readouts. In addition, molecular testing for gonorrhoea is often coupled with chlamydia; however, due to the complexity of culturing obligate intracellular bacterium "Chlamydia trachomatis" in the laboratory, we were unable to examine how sample storage parameters impact the testing sensitivity of "C. trachomatis." A limitation of this study is that tested samples lacked a background clinical matrix, and thus cannot be directly extrapolated to the storage of clinical samples. In clinical samples, host and commensal genomic material, including nucleases, and DNA extraction and amplification inhibitors can potentially affect sample integrity and nucleic acid amplification test performance.\textsuperscript{8} Furthermore, the anatomical site of sample collection may impact on the composition of the clinical matrix and microbial load.\textsuperscript{9} In conclusion, our data demonstrate that regardless of variations in sample storage parameters, in particular time and temperature, "N. gonorrhoeae" samples can be accurately detected across molecular diagnostic platforms. This work provides a premise for service providers, and by extension diagnostic laboratories to offer more accessible STI testing, specifically within the self-collection framework.

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Contributors JAF, MK, DW and SP designed the study. JAF and MK performed technical and statistical analysis. FA assisted with data generation. JAF prepared the figures and JAF, JP, DW and FA prepared the manuscript which was reviewed by all authors. All authors had full access to all the data in the study and had full responsibility for the decision to submit the manuscript. SP is the guarantor for the manuscript.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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REFERENCES


SUPPLEMENTARY MATERIAL

Evaluating pre-analytical sample storage parameters for nucleic acid-based detection of *Neisseria gonorrhoeae*
SUPPLEMENTARY TABLE 1. In-house quantitative PCR (qPCR) assay designed for *N. gonorrhoeae* detection of the *porA* pseudogene.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Required volume per reaction</th>
</tr>
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<tr>
<td>2x Ex Taq qPCR premix (Cat. ID RR390A)</td>
<td>10 μL</td>
</tr>
<tr>
<td>Nuclease free water</td>
<td>6.5 μL</td>
</tr>
<tr>
<td>papTM probe (5.6 μM)</td>
<td>0.5 μL</td>
</tr>
<tr>
<td>papTM sense primer (12 μM)</td>
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</tr>
<tr>
<td>papTM antisense primer (12 μM)</td>
<td>0.5 μL</td>
</tr>
<tr>
<td>Template genomic DNA</td>
<td>2 μL</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>20 μL</strong></td>
</tr>
</tbody>
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SUPPLEMENTARY FIGURE PANEL 1. Comparing the analytical sensitivity of *N. gonorrhoeae* samples stored in generic (Viral Transport Medium, & Amies Liquid Media) and commercial (Aptima Multitest Swab Transport Media, & Abbott Alinity transport buffer) buffers. All samples represented were of high starting *N. gonorrhoeae* concentration and processed using the Alinity m STI assay. (A) Samples stored at -20°C. (B) Samples stored at 4°C. (C) Samples stored at 25°C. (D) Samples stored at 37°C. (E) Samples stored for 2 days. (F) Samples stored for 4 days. (G) Samples stored for 7 days. (H) Samples stored for 14 days. (I) Samples stored for 30 days. Unless noted otherwise, mean cycle threshold (Ct) value for three biological replicates are visualised as points together with one standard deviation as error bars. * p < 0.05, ** p < 0.01 relative to Day 0 25°C baseline samples.
SUPPLEMENTARY FIGURE PANEL 2. Comparing the analytical sensitivity of *N. gonorrhoeae* samples stored in generic (Viral Transport Medium, & Amies Liquid Media) and commercial (Aptima Multitest Swab Transport Media, & Abbott Alinity transport buffer) buffers. All samples represented were of middle starting *N. gonorrhoeae* concentration and processed using the Alinity m STI assay. (A) Samples stored at -20°C. (B) Samples stored at 4°C. (C) Samples stored at 25°C. (D) Samples stored at 37°C. (E) Samples stored for 2 days. (F) Samples stored for 4 days. (G) Samples stored for 7 days. (H) Samples stored for 14 days. (I) Samples stored for 30 days. Unless noted otherwise, mean cycle threshold (Ct) value for three biological replicates are visualised as points together with one standard deviation as error bars. * p < 0.05, ** p < 0.01 relative to Day 0 25°C baseline samples.
Supplemental material

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A

B

C

D

E

F

G

H

I

Buffers — Viral Transport Medium
Amies Liquid Media
Aptima Multitest Swab Transport Media
Abbott Alinity transport buffer
SUPPLEMENTARY FIGURE PANEL 3. Comparing the analytical sensitivity of *N. gonorrhoeae* samples stored in generic (Viral Transport Medium, & Amies Liquid Media) and commercial (Aptima Multitest Swab Transport Media, & Abbott Alinity transport buffer) buffers. All samples represented were of low starting *N. gonorrhoeae* concentration and processed using the Alinity m STI assay. (A) Samples stored at -20°C. (B) Samples stored at 4°C. (C) Samples stored at 25°C. (D) Samples stored for 37°C. (E) Samples stored for 2 days. (F) Samples stored for 4 days. (G) Samples stored for 7 days. (H) Samples stored for 14 days. (I) Samples stored for 30 days. Unless noted otherwise, mean cycle threshold (Ct) value for three biological replicates are visualised as points together with one standard deviation as error bars. *p < 0.05, **p < 0.01 relative to Day 0 25°C baseline samples.
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SUPPLEMENTARY FIGURE PANEL 4. Comparing the analytical sensitivity of *N. gonorrhoeae* samples stored in generic buffers, Viral Transport Medium and Amies Liquid Media. All samples represented were of high starting *N. gonorrhoeae* concentration and processed using the Xpert CT/NG assay – NG2 target represented. (A) Samples stored at -20°C. (B) Samples stored at 4°C. (C) Samples stored at 25°C. (D) Samples stored at 37°C. (E) Samples stored for 2 days. (F) Samples stored for 4 days. (G) Samples stored for 7 days. (H) Samples stored for 14 days. (I) Samples stored for 30 days. Unless noted otherwise, mean cycle threshold (Ct) value for three biological replicates are visualised as points together with one standard deviation as error bars. * p < 0.05, ** p < 0.01 relative to Day 0 25°C baseline samples.
SUPPLEMENTARY FIGURE PANEL 5. Comparing the analytical sensitivity of *N. gonorrhoeae* samples stored in generic buffers, Viral Transport Medium and Amies Liquid Media. All samples represented were of middle starting *N. gonorrhoeae* concentration and processed using the Xpert CT/NG assay – NG2 target represented.

(A) Samples stored at -20°C. (B) Samples stored at 4°C. (C) Samples stored at 25°C. (D) Samples stored at 37°C. (E) Samples stored for 2 days. (F) Samples stored for 4 days. (G) Samples stored for 7 days. (H) Samples stored for 14 days. (I) Samples stored for 30 days. Unless noted otherwise, mean cycle threshold (Ct) value for three biological replicates are visualised as points together with one standard deviation as error bars. * p < 0.05, ** p < 0.01 relative to Day 0 25°C baseline samples.
SUPPLEMENTARY FIGURE PANEL 6. Comparing the analytical sensitivity of *N. gonorrhoeae* samples stored in generic buffers, Viral Transport Medium and Amies Liquid Media. All samples represented were of low starting *N. gonorrhoeae* concentration and processed using the Xpert CT/NG assay – NG2 target represented.

(A) Samples stored at -20°C. (B) Samples stored at 4°C. (C) Samples stored at 25°C. (D) Samples stored at 37°C. (E) Samples stored for 2 days. (F) Samples stored for 4 days. (G) Samples stored for 7 days. (H) Samples stored for 14 days. (I) Samples stored for 30 days. Unless noted otherwise, mean cycle threshold (Ct) value for three biological replicates are visualised as points together with one standard deviation as error bars. * p < 0.05, ** p < 0.01 relative to Day 0 25°C baseline samples.
SUPPLEMENTARY FIGURE PANEL 7. Comparing the analytical sensitivity of *N. gonorrhoeae* samples stored in generic (Viral Transport Medium, & Amies Liquid Media) and commercial (Aptima Multitest Swab Transport Media, & Abbott Alinity transport buffer) buffers. All samples represented were of high starting *N. gonorrhoeae* concentration and processed using an in-house qPCR (*porA*) assay. (A) Samples stored at -20°C. (B) Samples stored at 4°C. (C) Samples stored at 25°C. (D) Samples stored at 37°C. (E) Samples stored for 2 days. (F) Samples stored for 4 days. (G) Samples stored for 7 days. (H) Samples stored for 14 days. (I) Samples stored for 30 days. Unless noted otherwise, mean cycle threshold (Ct) value for three biological replicates are visualised as points together with one standard deviation as error bars. * p < 0.05, ** p < 0.01 relative to Day 0 25°C baseline samples.
SUPPLEMENTARY FIGURE PANEL 8. Comparing the analytical sensitivity of *N. gonorrhoeae* samples stored in generic (Viral Transport Medium, & Amies Liquid Media) and commercial (Aptima Multitest Swab Transport Media, & Abbott Alinity transport buffer) buffers. All samples represented were of middle starting *N. gonorrhoeae* concentration and processed using an in-house qPCR (*porA*) assay. (A) Samples stored at -20°C. (B) Samples stored at 4°C. (C) Samples stored at 25°C. (D) Samples stored at 37°C. (E) Samples stored for 2 days. (F) Samples stored for 4 days. (G) Samples stored for 7 days. (H) Samples stored for 14 days. (I) Samples stored for 30 days. Unless noted otherwise, mean cycle threshold (Ct) value for three biological replicates are visualised as points together with one standard deviation as error bars. * p < 0.05, ** p < 0.01 relative to Day 0 25°C baseline samples.
SUPPLEMENTARY FIGURE PANEL 9. Comparing the analytical sensitivity of *N. gonorrhoeae* samples stored in generic (Viral Transport Medium, & Amies Liquid Media) and commercial (Aptima Multitest Swab Transport Media, & Abbott Alinity transport buffer) buffers. All samples represented were of low starting *N. gonorrhoeae* concentration and processed using an in-house qPCR (*porA*) assay. (A) Samples stored at -20°C. (B) Samples stored at 4°C. (C) Samples stored at 25°C. (D) Samples stored at 37°C. (E) Samples stored for 2 days. (F) Samples stored for 4 days. (G) Samples stored for 7 days. (H) Samples stored for 14 days. (I) Samples stored for 30 days. Unless noted otherwise, mean cycle threshold (Ct) value for three biological replicates are visualised as points together with one standard deviation as error bars. * p < 0.05, ** p < 0.01 relative to Day 0 25°C baseline samples.
SUPPLEMENTARY TABLE 2A. *N. gonorrhoeae* detection for high concentration samples processed using the Aptima Combo 2 assay.

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<th>Day 2</th>
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<th>Day 7</th>
<th>Day 14</th>
<th>Day 30</th>
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<td>-20°C</td>
<td>3/3 (100%)</td>
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<td>3/3 (100%)</td>
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<tr>
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SUPPLEMENTARY TABLE 2B. *N. gonorrhoeae* detection for middle concentration samples processed using the Aptima Combo 2 assay.

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<th>Day 14</th>
<th>Day 30</th>
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<td>3/3 (100%)</td>
<td>1/1 (100%)</td>
<td>3/3 (100%)</td>
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<td>3/3 (100%)</td>
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</tr>
<tr>
<td>37°C</td>
<td>2/2 (100%)</td>
<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
<td>3/3 (100%)</td>
<td>3/3 (100%)</td>
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SUPPLEMENTARY TABLE 2C. *N. gonorrhoeae* detection for low concentration samples processed using the Aptima Combo 2 assay.

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<th>Day 14</th>
<th>Day 30</th>
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<td>3/3 (100%)</td>
<td>2/2 (100%)</td>
<td>3/3 (100%)</td>
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<tr>
<td>4°C</td>
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<td>1/1 (100%)</td>
<td>3/3 (100%)</td>
<td>2/2 (100%)</td>
</tr>
<tr>
<td>25°C</td>
<td>3/3 (100%)</td>
<td>2/2 (100%)</td>
<td>2/2 (100%)</td>
<td>3/3 (100%)</td>
<td>2/2 (100%)</td>
</tr>
<tr>
<td>37°C</td>
<td>2/2 (100%)</td>
<td>N/A*</td>
<td>1/1 (100%)</td>
<td>3/3 (100%)</td>
<td>2/2 (100%)</td>
</tr>
</tbody>
</table>

* All triplicates encountered instrumental errors during processing