Clinical Performance Comparison of Configurable Versus Fixed-Panel Multiplex PCR for Sexually Transmitted Infection Diagnostics

Martin Horlitz[1], Erika Czyžová[2], Petra Švástová[1], Soňa Čejková[1], Kateřina Pokorná[1]

[1] GeneProof a.s., Vídeňská 101/119, 619 00 Brno, Czech Republic [2] Nemocnice Šumperk a.s., MKB laboratoř, Nerudova 640/41, 787 01 Šumperk, Czech Republic

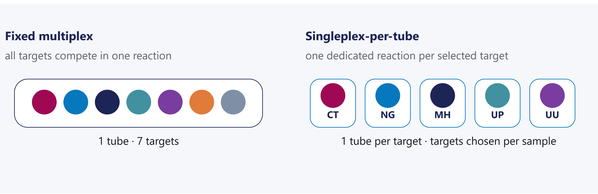
GeneProof® part of NuvinkaDx

AMP 2026 · Poster: ID011 · Abstract: 2465443

INTRODUCTION

- Multiplex PCR assays for sexually transmitted infection (STI) diagnostics may exhibit competitive amplification interference: when a high-copy and a low-copy target co-occur in one specimen, sensitivity for the low-copy target can be reduced.
- Singleplex-per-tube assays dedicate one reaction to each target and may therefore preserve sensitivity for low-abundance pathogens.
- A configurable panel additionally allows the target set to be selected per sample, instead of running a fixed multiplex for every specimen.

Aim: compare clinical and analytical performance of the GeneProof myCROBE STI panel (configurable, singleplex-per-tube) with Seegene Allplex STI Essential (fixed 7-plex) using paired testing of the same samples.



MATERIALS AND METHODS

Dataset 1 – Clinical Specimens

155 residual urogenital specimens
Hospital Šumperk, CZ · 11 Mar – 9 Apr 2026

→ **Seegene Allplex STI Essential**
fixed 7-plex real-time PCR

→ **GeneProof myCROBE STI panel**
configurable singleplex-per-tube PCR

→ **Paired comparison**
qualitative agreement · Ct offset · ΔCt between co-detected targets

Targets compared: CT – Chlamydia trachomatis, NG – Neisseria gonorrhoeae, MH – Mycoplasma hominis, UP – Ureaplasma parvum, UU – Ureaplasma urealyticum.

Same nucleic-acid extract / specimen tested in parallel on both workflows.

Samples were processed and analyzed according to the manufacturer's instructions for the Seegene Allplex STI Essential and GeneProof myCROBE STI Panel, respectively.

Dataset 2 – Spike in Specimens

Samples 1–5
high-copy CT (expected Ct 17–25) + low-copy NG (expected Ct 30–32)

Samples 6–10
high-copy NG (expected Ct 18–25) + low-copy CT (expected Ct 30–32)

10 STI-negative vaginal swabs spiked with CT and NG material and tested on both platforms, directly challenging low-copy detection in the presence of a high-copy co-target.

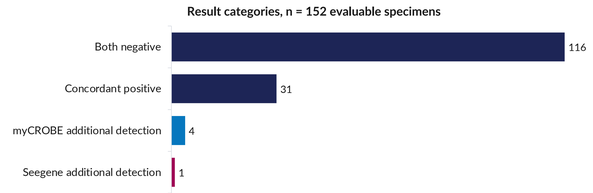
Definitions

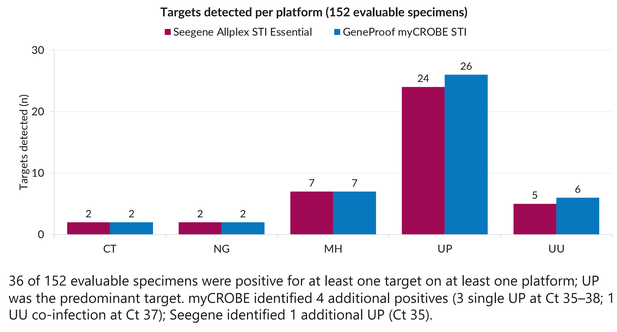
- Evaluable specimen: valid result on both platforms. myCROBE target signals with internal-control failure were classified invalid and excluded.
- Concordance: identical target calls on both platforms. Ct offset = Ct(myCROBE) − Ct(Seegene) for targets detected by both.
- ΔCt spread = highest − lowest Ct among co-detected targets within one specimen.
- Note: each workflow was run on its own PCR instrument type with the manufacturer's analysis software and settings; Ct values are therefore not directly comparable across platforms.

RESULTS 1 · CLINICAL AGREEMENT (DATASET 1)

96.7%

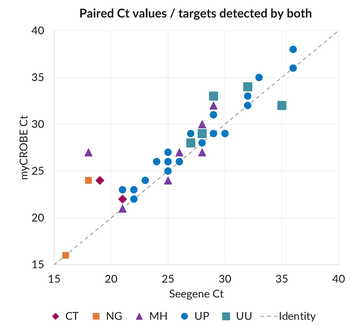
Overall Agreement
147 of 152 evaluable specimens (116 double-negative + 31 concordant-positive). 3 of 155 specimens excluded because of invalid myCROBE UP results.

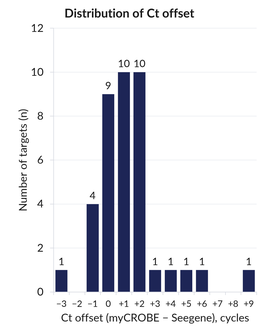




36 of 152 evaluable specimens were positive for at least one target on at least one platform; UP was the predominant target. myCROBE identified 4 additional positives (3 single UP at Ct 35–38; 1 UU co-infection at Ct 37); Seegene identified 1 additional UP (Ct 35).

RESULTS 2 · PAIRED Ct COMPARISON (DATASET 1)



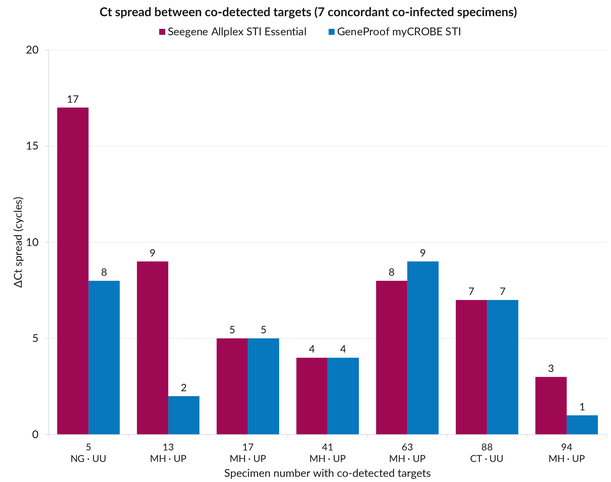


- Ct values of the two platforms were well correlated across all five targets; median Ct offset (myCROBE − Seegene) was +1 cycle and 33/39 targets (85%) were within ±2 cycles.
- Per-target median offsets: CT +3 (n = 2), NG +3 (n = 2), MH +1 (n = 7), UP +1 (n = 23), UU +1 (n = 5).
- Absolute Ct values are not directly comparable between workflows: PCR instrument-type-specific characteristics (optics, thermal profile) and differences in analysis-software settings (baseline/threshold), together with sampling variation between paired aliquots, may partially account for the observed offset.

RESULTS 3 · DISCORDANT & CO-INFECTED SPECIMENS

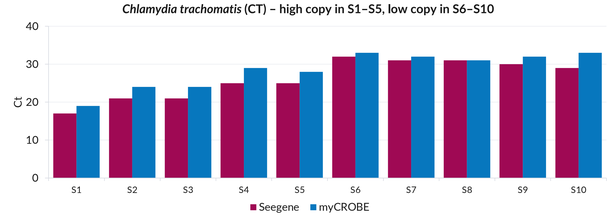
Sample	Seegene result (Ct)	myCROBE result (Ct)	Classification
34	negative	UP 35	myCROBE additional detection
58	negative	UP 38	myCROBE additional detection
127	negative	UP 35	myCROBE additional detection
145	UP 29	UP 29 + UU 37	myCROBE additional UU co-infection
118	UP 35	negative	Seegene additional detection
18	MH 36 · UU 33 · UP 29	MH 36 · UU 34 UP invalid (Ct 27.5)	excluded – UP internal-control failure
21	UP 20	UP invalid (Ct 21.1)	excluded – UP internal-control failure
24	UP 27	UP invalid (Ct 26.3)	excluded – UP internal-control failure

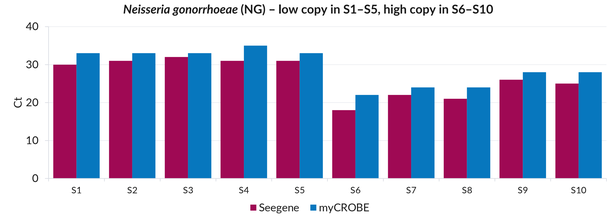
Table 1. All non-concordant and invalid specimens (**Dataset 1**). Discordant calls were low-copy UP/UU detections (Ct 35–38). The three invalid myCROBE results showed clear UP amplification (Ct 21–27) but a failed internal control in the UP reaction, while Seegene called UP at Ct 20–27 in the same specimens.



- All co-infecting targets in the 7 concordant co-infected specimens were detected by both platforms; the Ct spread between co-detected targets ranged from 3 to 17 cycles with Seegene and from 1 to 9 cycles with myCROBE.
- The different spread was not associated with loss of any minor target and reflects Ct differences for individual targets rather than a difference in qualitative performance.
- The largest between-platform differences (specimens 5 and 13) involved the dominant high-copy target (Seegene Ct 18) and were accompanied by concordant detection of the minor target.
- In specimen 145, myCROBE detected an additional low-copy UU (Ct 37) alongside a concordant UP (Ct 29).
- Total co-infected specimens: 7 concordant, 1 myCROBE-only UU co-detection (#145) and 1 triple infection excluded for an invalid UP result (#18).

RESULTS 4 · SPIKE-IN CO-TARGETS (DATASET 2)





Target	Detected (Seegene / myCROBE)	Low-copy Ct range (SG / MC)	Median offset MC − SG (range)
CT	10 / 10	29–32 / 31–33	+2.5 (0 to +4)
NG	10 / 10	30–32 / 33–35	+2.5 (+1 to +4)

- All 10 high-copy and all 10 low-copy spiked targets were detected by both platforms (20/20) – no low-copy target was lost in the presence of a high-copy co-target on either platform.
- A consistent Ct offset (myCROBE − Seegene, median +2.5 cycles for CT and NG) was observed, in line with Dataset 1 and compatible with instrument- and software-related differences between the workflows.
- Incidental UP (6 swabs) and MH (2 swabs) present in the swab matrix were detected by both platforms with Ct within ±4 cycles.

SUMMARY AND CONCLUSIONS

- The configurable singleplex-per-tube myCROBE STI panel and the fixed 7-plex Seegene Allplex STI Essential showed 96.7% overall agreement in 152 evaluable clinical specimens.
- myCROBE demonstrated higher sensitivity for low-copy UP (3 additional single detections, Ct 35–38) and for a low-copy UU co-infection (Ct 37); Seegene detected one additional UP (Ct 35).
- myCROBE produced internal-control failures in 3 clearly UP-positive specimens (Ct 21–27), resulting in invalid results – a limitation requiring follow-up.
- Ct values and ΔCt between co-detected targets differed modestly between platforms (median offset +1 cycle clinical, +2.5 cycles spike-in) and do not indicate a difference in qualitative performance (see note below).
- In spike-in samples, both platforms detected low-copy CT/NG in the presence of a high-copy co-target; no competitive loss of the minor target was observed at the tested levels.
- These findings highlight performance trade-offs between fixed and configurable multiplex STI platforms. A flexible singleplex PCR system may offer clinical utility advantages, but a sensitivity advantage for low-positive targets must be confirmed in larger clinical cohorts.

Note on Ct comparability. Any observed Ct offset or ΔCt difference between the platforms may be partially influenced by PCR instrument-type-specific differences (optical detection, thermal characteristics) and by differences in analysis-software settings (baseline and threshold algorithms).

Regulatory disclaimer. GeneProof a.s. products referenced on this poster are CE-marked in vitro diagnostic medical devices (CE-IVD) available in the EU and other countries recognizing the CE mark. They have not been cleared or approved by the U.S. FDA and are not available for in vitro diagnostic use in the United States. Product availability varies by country. All trademarks are the property of their respective owners.

Corresponding author: Martin Horlitz, GeneProof a.s., Brno, Czech Republic | Disclosures: M.H., P.Š., S.Č. and K.P. are employees of GeneProof a.s.; E.C. declares no conflict of interest. | AMP 2026 Annual Meeting & Expo, Seattle, WA

www.geneproof.com