JCM Accepted Manuscript Posted Online 3 March 2021
J Clin Microbiol doi:10.1128/JCM.00089-21
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2 **Reflex detection of ciprofloxacin resistance in *Neisseria gonorrhoeae***

3 **using the SpeeDx ResistancePlus® GC assay**

4

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27 **Running title:** Molecular detection of ciprofloxacin-resistant gonorrhoea

28 **Keywords:** *Neisseria gonorrhoeae*; molecular diagnostic; antimicrobial resistance; whole

29 genome sequencing

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31 **Word count (main text): 1604**

32 **Abstract word count: 79**

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51 **ABSTRACT**

52 Resistance-guided therapy (RGT) for gonorrhoea may reduce unnecessary use of broad-
53 spectrum antibiotics. When reflexed from the Aptima Combo 2 assay, the
54 ResistancePlusGC® assay demonstrated 94.8% sensitivity and 100.0% specificity for
55 *Neisseria gonorrhoeae* detection. Of the 379 concordant *N. gonorrhoeae*-positive samples,
56 86.8% were found to possess the *gyrA* S91F mutation, which was highly predictive for
57 ciprofloxacin resistance and stable across 3,144 publicly available *N. gonorrhoeae* genomes.
58 Our work supports the feasibility of implementing RGT for gonorrhoea into routine
59 molecular workflows.

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77 **INTRODUCTION**

78 The increasing incidence of gonorrhoea globally is a major public health threat. For decades,
79 gonorrhoea has been treated before antibiotic susceptibility results are known, according to
80 local treatment guidelines which are generally based on the local prevalence of antimicrobial
81 resistance (AMR). In many settings, dual empiric therapy with oral azithromycin (1g) and
82 intramuscular ceftriaxone (500mg) is recommended (1). With recent reports of increasing
83 resistance, individualized therapy based on the molecular detection of resistance determinants
84 (resistance-guided therapy; RGT) has been suggested as a way of improving antimicrobial
85 stewardship and delaying the emergence of AMR (2, 3). In particular, RGT for ciprofloxacin
86 has been incorporated into gonorrhoea treatment guidelines in the United Kingdom and was
87 shown to be feasible and effective in a recent multisite clinical study in the United States (4,
88 5).

89

90 Ciprofloxacin resistance in *N. gonorrhoeae* occurs predominantly through point mutations in
91 the DNA gyrase A gene (*gyrA*), most commonly a single point mutation at the Serine 91
92 codon (GyrA S91F), which is highly predictive of ciprofloxacin resistance. Other mutations
93 associated with increased ciprofloxacin minimum inhibitory concentrations (MICs) include a
94 point mutation at the Aspartic Acid 95 codon of *gyrA* (D95), and mutations in the
95 topoisomerase IV *parC* gene (6). The highly recombinogenic nature of *N. gonorrhoeae*
96 means that continuous surveillance is critical to ensure the ongoing utility of diagnostic
97 markers used for RGT and to detect 'diagnostic escape variants' (2). Accordingly, the aims of
98 this study were: (i) to assess the performance characteristics of a recently introduced
99 commercial assay for ciprofloxacin RGT against a widely used nucleic acid amplification test
100 (NAAT) for *N. gonorrhoeae*, and (ii) to determine the genomic stability of molecular

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101 determinants of ciprofloxacin resistance in a large collection of publicly available
102 *N. gonorrhoeae* genomes.

103 **METHODS**

104 Clinical samples were obtained from Melbourne Sexual Health Centre (MSHC), the largest
105 public sexual health service in Melbourne, Australia. 445 clinical samples collected from
106 March to May 2019 were stored at room temperature in Hologic® Aptima Unisex Specimen
107 Transport Tubes (Hologic, San Diego, CA, USA) as per manufacturer's instructions and
108 tested for *Chlamydia trachomatis* and *N. gonorrhoeae* using the Aptima Combo 2 assay
109 (AC2; Hologic, San Diego, CA, USA) within 24 hours. Where available, consecutive clinical
110 samples were collected. In the AC2 assay, transcription-mediated amplification (TMA) of
111 diagnostic targets results in the production of luminescent signals, quantified as relative light
112 units (RLUs). The RLU value is then used to categorise results as positive, negative and
113 equivocal (7).

114

115 In total, 400 µL of remnant AC2 samples underwent DNA extraction using the
116 QIASymphony™ DSP Virus/Pathogen Midi Kit Complex 400 protocol, as per
117 manufacturer's instructions (Qiagen, Hilden, Germany). These samples were stored as per
118 manufactures instructions at room temperature prior to testing on the Aptima assay, and were
119 all tested within fourteen days post-NAAT. PCR testing for *N. gonorrhoeae* and the GyrA
120 S91F mutation was performed on 5 µL of extracted DNA using the previously described
121 ResistancePlus® GC assay (SpeeDx Pty Ltd, Sydney, Australia) (8, 9) on a LightCycler ®
122 480 II (LC480 II; Roche, Switzerland). Briefly, the assay reports detection across five
123 channels using the following targets: (i) detection of the *N. gonorrhoeae opa* gene; (ii)
124 detection of the *N. gonorrhoeae porA* gene; (iii) detection of *gyrA* S91 (wild type); (iv)
125 detection of *gyrA* S91F and (v) an internal control to monitor extraction efficiency and qPCR

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126 inhibition. Interpretation of the results was performed using the ResistancePlus® GC (7500)
127 analysis software. The assay reports the following results: (i) whether *N. gonorrhoeae* was
128 detected or not detected, and (ii) if *N. gonorrhoeae* was detected, whether *gyrA* is wild type, a
129 *gyrA* S91F mutation or indeterminant. Statistical analyses were conducted using GraphPad
130 Prism (version 8.4.3). Binomial 95% confidence intervals (CI) were calculated for all
131 proportions. Differences between groups were calculated using either a Mann-Whitney test or
132 chi-square test. Bioinformatic analyses are described in the Supplementary Appendix.
133
134 **RESULTS AND DISCUSSION**
135 ***Assessment of the ResistancePlus® GC assay for detection of Neisseria gonorrhoeae***
136 In total, 445 clinical samples from different anatomical sites (from 336 patients) were tested
137 using the ResistancePlus® GC assay (400 *N. gonorrhoeae* NAAT-positive and 45 *N.*
138 *gonorrhoeae* NAAT-negative Aptima samples) (Table 1). In total 97/336 (28.9%) patients
139 had samples from ≥1 anatomical site. Compared to Aptima NAAT, the overall sensitivity
140 and specificity of the ResistancePlus® GC assay for detection of *N. gonorrhoeae* was 94.8%
141 (379/400; 95% CI: 92.6% - 97.0%) and 100% (45/45; 95% CI: 97.8% - 100.0%),
142 respectively. There was a significant difference in RLU values as reported by the AC2 assay
143 between detected *N. gonorrhoeae*-positive samples (median RLU 1536, inter-quartile range
144 (IQR) 1453 – 1583 RLUs) on the ResistancePlus® GC assay compared to
145 undetected/discordant samples (median RLU 863, IQR 455 – 1205 RLUs, $P < 0.001$), which
146 may suggest a lower bacterial load in negative samples.
147
148 ***Identification of gyrA alleles***
149 The ResistancePlus® GC assay successfully generated a *gyrA* result in 329 (86.8%) of 379
150 samples that were positive for *N. gonorrhoeae* by AC2 (Table 2). Of these, 206/329 (62.6%)

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151 samples had a *gyrA* S91 wild type (WT) result, and 123/329 (37.4%) had a *gyrA* S91F
152 mutation. The remaining 50/329 (15.2%) *N. gonorrhoeae* positive samples were
153 indeterminate for *gyrA* (i.e. the ResistancePlus® GC assay could not determine whether a
154 WT or mutant *gyrA* was present). Given the samples were found to be *N. gonorrhoeae*
155 positive by the AC2 and ResistancePlus® GC assay it is unlikely the indeterminate result was
156 due to cross-reactivity with non-gonococcal strains. Instead, it is likely that the *gyrA*
157 detection sensitivity is lower than *N. gonorrhoeae* detection. This is consistent with previous
158 work by Cotton *et al*. 2020 that reported a sensitivity of 97.1% for detection of *gyrA* and a
159 sensitivity of 98.5% for detection of *N. gonorrhoeae* (10). In our study, samples with
160 indeterminate *gyrA* results had significantly lower AC2 reported RLU values compared to
161 samples with *gyrA* detected (median RLU 1091 vs 1536, $P < 0.001$) (Figure 1; Table S1).
162 Indeterminate *gyrA* results were significantly more likely in anorectal samples (28/150;
163 18.7%) and pharyngeal samples (20/176; 11.4%) compared to urogenital sites (2/74; 2.7%, P
164 < 0.001) (Figure 2). A limitation of our study was the lack of associated phenotypic data; this
165 limitation reflects the increasing use of molecular testing, which reduces the availability of
166 isolates for additional analyses.
167
168 ***Genomic assessment of gyrA S91F across Neisseria gonorrhoeae lineages***
169 The utility of the ResistancePlus® GC assay in RGT depends on the relative frequency and
170 locations of mutations across lineages of ciprofloxacin resistance mutations, particularly *gyrA*
171 S91F. A collection of 8,179 non-duplicated (one isolate per individual) *N. gonorrhoeae*
172 global isolates with available minimum inhibitory concentration (MIC) data were obtained
173 from Pathogenwatch (11), including isolates from Victoria, Australia (12). Of these, 3,144
174 isolates were phenotypically resistant to ciprofloxacin and were examined for mutations in
175 *gyrA* and *parC*. Isolates were defined as resistant to ciprofloxacin if the MIC was ≥ 1 μg/mL

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176 as per Clinical and Laboratory Standards Institute (CLSI) guidelines (13) (Supplementary
177 Appendix, Table S2). In total, 3,100/3,144 (98.6%) of isolates identified as phenotypically
178 ciprofloxacin-resistant had the S91F mutation and 108/5,035 (2.1%) of isolates identified as
179 phenotypically ciprofloxacin-susceptible had the S91F mutation. Accordingly, the sensitivity
180 and specificity of the *gyrA* S91F mutation for conferring ciprofloxacin resistance in
181 *N. gonorrhoeae* isolates was 98.6% and 97.9%, respectively. In addition, 3,095/3,100
182 (99.8%) of isolates with an S91F mutation also harboured a D95 mutation, most commonly
183 D95G (1,996/3,095; 64.5%), D95A (954/3,095; 30.8%) and D95N (145/3,095; 4.7%).
184 Further, mutations in *parC* were identified in 2,573/3,144 (81.8%) of ciprofloxacin-resistant
185 *N. gonorrhoeae*. These included mutations at S87 (1,964/2,573; 76.3%), D86 (596/2,573;
186 23.2%), S88 (106/2,573; 4.1%), and E91 (49/2,573; 1.9%). Of the 44/3,144 (1.4%)
187 ciprofloxacin-resistant *N. gonorrheoae* isolates that did not have a *gyrA* S91F mutation, 2/44
188 (4.6%) carried a *parC* mutation (1: D86N, 1: S87R), with no other *gyrA* or *parC* mutations
189 identified in the remaining isolates. Ciprofloxacin-resistant isolates with a *gyrA* S91F
190 mutation were identified across 16 multi-locus sequence types (STs) (Supplementary
191 Appendix, Table S2), where the dominant STs were ST1901 (945/3,144; 30.1%) and ST7363
192 (425/3,144; 13.5%). One limitation of our approach was that we relied on phenotypic data
193 reported by other studies, although we applied CLSI criteria for ciprofloxacin resistance to all
194 isolates to enable a standardised comparison.
195

196 **CONCLUSIONS**

197 In summary, we evaluated the sensitivity and specificity for detection of *N. gonorrhoeae*
198 using a commercially available ciprofloxacin RGT assay, with positive results more likely
199 using samples with higher RLU values on the AC2 assay. The bacterial load of *N.*
200 *gonorrhoeae* varies between anatomical sites and may therefore affect sensitivity of the assay

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201 (14). We also found that indeterminate *gyrA* results were more likely at lower RLUs from
202 extragenital sites, possibly suggesting a lower bacterial load in these samples and/or potential
203 cross-reactivity with non-gonococcal *Neisseria* isolates. This 'therapeutic gap' (i.e. positive
204 for *N. gonorrhoeae* using TMA and negative for *N. gonorrhoeae* and/or *gyrA* indeterminate
205 using the ResistancePlusGC® assay) is likely due to the differential analytical sensitivity
206 between the TMA-based and PCR-based assays. Although this only constituted a minority of
207 samples in our study (15.2%), in clinical practice this would mean a proportion of patients
208 would have treatment with empiric rather than 'tailored' therapy. Importantly, the clinical
209 and economic utility of resistance-guided therapy for ciprofloxacin is likely to vary based on
210 local rates of ciprofloxacin resistance. Finally, we found that the GyrA S91F mutation was
211 both highly predictive for ciprofloxacin resistance and stable across a range of *N.*
212 *gonorrhoeae* lineages from multiple geographic settings. Collectively, our work further
213 supports the feasibility of implementing RGT for gonorrhoea into routine molecular testing.
214 Future work should explore improved integration of assays for RGT into large-scale NAAT
215 workflows for gonorrhoea and monitor clinical outcome data in patients treated using RGT.
216

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217 **FUNDING**

218 This study was supported by a Project Grant from the National Health and Medical Research
219 Council (NHMRC) of Australia (GNT1147735; DAW; MYC; EPFC; CKF). E.P.F.C. and
220 D.A.W. are supported by an Australian National Health and Medical Research Council
221 Emerging Leadership Investigator Grants (GNT1172873 and GNT1174555, respectively).
222 C.K.F. and C.S.B. are supported by an NHMRC Leadership Investigator Grant
223 (GNT1172900 and GNT1173361, respectively). B.J.H. is supported by an NHMRC
224 Practitioner Fellowship (GNT1105905).

225

226 **ETHICS**

227 This study was approved by the South Eastern Sydney Local Health District Human Research
228 Ethics Committee (HREC/17/POWH/510).

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230 **REFERENCES**

231 1. Unemo M. 2015. Current and future antimicrobial treatment of gonorrhoea - the
232 rapidly evolving *Neisseria gonorrhoeae* continues to challenge. *BMC Infect Dis*
233 15:364.

234 2. Tuite AR, Gift TL, Chesson HW, Hsu K, Salomon JA, Grad YH. 2017. Impact of
235 Rapid Susceptibility Testing and Antibiotic Selection Strategy on the Emergence and
236 Spread of Antibiotic Resistance in Gonorrhea. *J Infect Dis* 216:1141-1149.

237 3. Trembizki E, Guy R, Donovan B, Kaldor JM, Lahra MM, Whiley DM. 2016. Further
238 evidence to support the individualised treatment of gonorrhoea with ciprofloxacin.
239 *Lancet Infect Dis* 16:1005-1006.

240 4. Klausner JD, Bristow CC, Soge OO, Shahkolahi A, Waymer T, Bolan RK, Philip SS,
241 Asbel LE, Taylor SN, Mena LA, Goldstein DA, Powell JA, Wierzbicki MR, Morris
242 SR. 2020. Resistance-Guided Treatment of Gonorrhea: A Prospective Clinical Study.
243 *Clin Infect Dis* doi:10.1093/cid/ciaa596.

244 5. Fifer H, Saunders J, Soni S, Sadiq ST, FitzGerald M. 2020. 2018 UK national
245 guideline for the management of infection with *Neisseria gonorrhoeae*. Int J STD
246 AIDS 31:4-15.

247 6. Unemo M, Shafer WM. 2014. Antimicrobial resistance in *Neisseria gonorrhoeae* in
248 the 21st century: past, evolution, and future. *Clin Microbiol Rev* 27:587-613.

249 7. Hologic Inc. 2016. Aptima Combo 2 Assay (Panther System). San Diego, CA USA.

250 8. Ebeyan S, Windsor M, Bordin A, Mhango L, Erskine S, Trembizki E, Mokany E, Tan
251 LY, Whiley D, Investigators GS. 2019. Evaluation of the ResistancePlus GC (beta)
252 assay: a commercial diagnostic test for the direct detection of ciprofloxacin
253 susceptibility or resistance in *Neisseria gonorrhoeae*. *J Antimicrob Chemother*
254 74:1820-1824.

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255 9. Allan-Blitz LT, Ellis OL, Wee R, Truong A, Ebeyan SM, Tan LY, Mokany E, Flynn
256 R, Klausner JD. 2019. Improved determination of *Neisseria gonorrhoeae* gyrase A
257 genotype results in clinical specimens. *J Antimicrob Chemother* 74:2913-2915.
258 10. Cotton S, McHugh MP, Etherson M, Shepherd J, Templeton KE. 2020. Evaluation of
259 the molecular detection of ciprofloxacin resistance in *Neisseria gonorrhoeae* by the
260 ResistancePlus GC assay (SpeeDx). *Diagn Microbiol Infect Dis* 99:115262.
261 11. Sánchez-Busó L, Yeats CA, Taylor B, Goater R, Underwood A, Abudahab K,
262 Argimón S, Ma KC, Mortimer TD, Cole MJ, Grad YH, Martin I, Raphael BH, Shafer
263 WM, Spiteri G, Town K, Wi T, Harris SR, Unemo M, Aanensen DM. 2020. A
264 community-driven resource for genomic surveillance of *Neisseria gonorrhoeae* at
265 Pathogenwatch. *bioRxiv* doi:10.1101/2020.07.03.186726.
266 12. Williamson DA, Chow EPF, Gorrie CL, Seemann T, Ingle DJ, Higgins N, Easton M,
267 Taiaroa G, Grad YH, Kwong JC, Fairley CK, Chen MY, Howden BP. 2019. Bridging
268 of *Neisseria gonorrhoeae* lineages across sexual networks in the HIV pre-exposure
269 prophylaxis era. *Nat Commun* 10:3988.
270 13. Clinical and Laboratory Standards Institute. 2020. M100 Performance Standards for
271 Antimicrobial Susceptibility Testing. Wayne, PA, USA.
272 14. Bissessor M, Tabrizi SN, Fairley CK, Danielewski J, Whitton B, Bird S, Garland S,
273 Chen MY. 2011. Differing *Neisseria gonorrhoeae* bacterial loads in the pharynx and
274 rectum in men who have sex with men: implications for gonococcal detection,
275 transmission, and control. *J Clin Microbiol* 49:4304-6.
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279 TABLE 1 Specimen type for clinical samples tested using both Aptima Combo 2 and

280 ResistancePlus® GC molecular assays.

Site	Sample Bank 1: *N. gonorrhoeae*-positive	Sample Bank 2: *N. gonorrhoeae*-negative
Anorectal	150 (37.5%)	15 (33.3%)
Pharyngeal	176 (44.0%)	22 (48.9%)
Urogenital	74 (18.5%)	8 (17.8%)
Total	400	45

281

282 Values are given as number of samples (percentages). Sample Bank 1 consisted of 400 *N.*

283 *gonorrhoeae* NAAT-positive clinical samples that were previously stored for 14 days at room

284 temperature in Hologic® Aptima Unisex Specimen Transport Tubes (Hologic, San Diego,

285 CA, USA) between 14th February to 29th May, 2019. Sample Bank 2 consisted of 45 *N.*

286 *gonorrhoeae* NAAT-negative clinical samples collected from routine gonorrhoea testing in

287 September 2019.

288

289 TABLE 2 ResistancePlus® GC results for the detection of *N. gonorrhoeae* and *gyrA* in

290 different anatomical sites of infection

Site	*N. gonorrhoeae* detected, *gyrA* indeterminate	*N. gonorrhoeae* detected, *gyrA* mutation detected	*N. gonorrhoeae* detected, *gyrA* mutation not detected	*N. gonorrhoeae* not detected	Total
Anorectal	28 (7.0%)	43 (10.8%)	70 (17.5%)	9 (2.2%)	150
Pharyngeal	20 (5.0%)	54 (13.5%)	94 (23.5%)	8 (2.0%)	176
Urogenital	2 (0.5%)	26 (6.5%)	42 (10.5%)	4 (1.0%)	74
Total	50	123	206	21	400

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292 Values are given as number of samples (percentages). Results for the 400 clinical *N.*

293 *gonorrhoeae* NAAT-positive samples as reported by SpeeDx ResistancePlus® GC assay.

294

295 **Figure 1. Detection of *Neisseria gonorrhoeae* and characterisation of *gyrA* using the**

296 **ResistancePlus® GC assay in relation to relative light units (RLUs) reported by the**

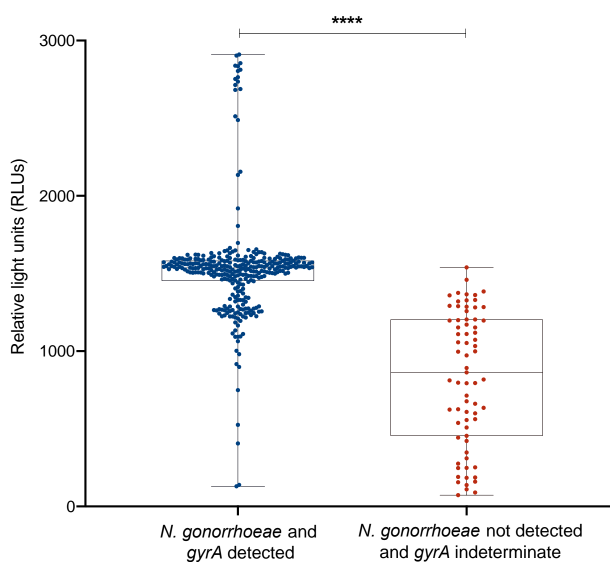
297 **Aptima Combo 2 assay.** Boxes depict the inter-quartile range, and the median is

298 represented by a short black line within the box. Whiskers represent the 5th and 95th

299 percentiles and dots represent individual samples. Statistically significant differences between

300 median RLUs are indicated with asterisks (**** $P < 0.0001$).

301



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304 **Figure 2. Detection of *Neisseria gonorrhoeae* and characterisation of *gyrA* in samples**
305 **derived from different anatomical sites of infection using the ResistancePlus® GC**
306 **assay.** Boxes depict the inter-quartile range, and the median is represented by a short black
307 line within the box. Whiskers represent the 5th and 95th percentiles and dots represent
308 individual samples. Statistically significant differences between median RLUs are indicated
309 with asterisks (**** $P < 0.0001$; ** $P < 0.01$). (A) NG detected and *gyrA* S91F mutant
310 detected; (B) NG detected and *gyrA* S91 WT detected; (C) NG detected and *gyrA*
311 indeterminate; (D) NG not detected.

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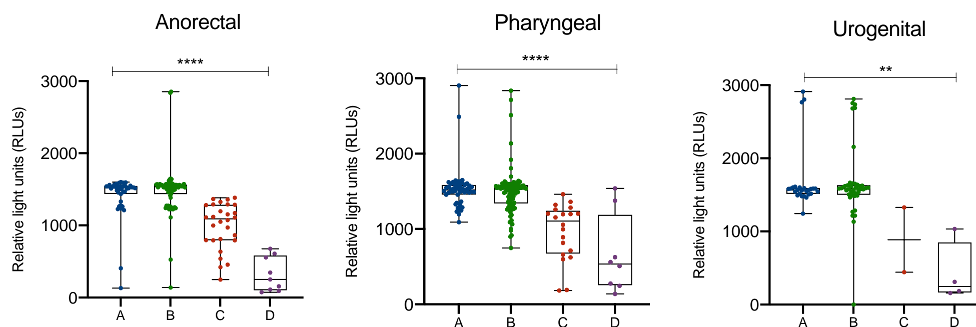

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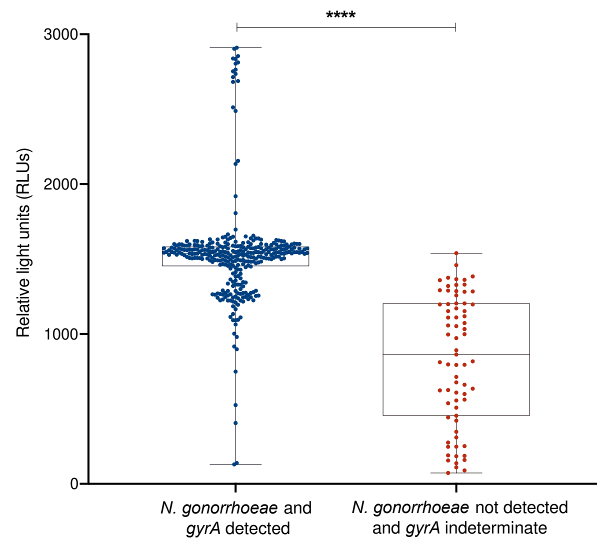

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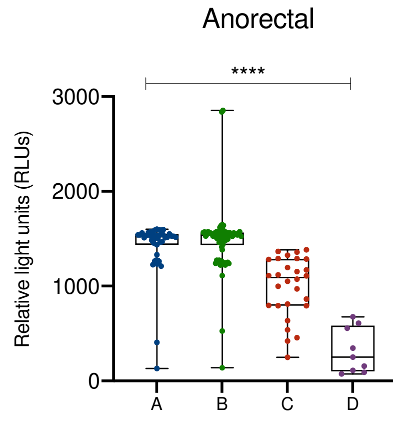

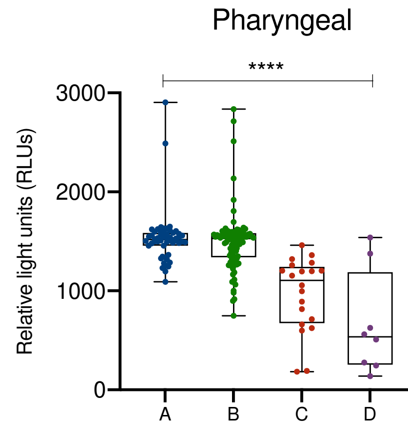

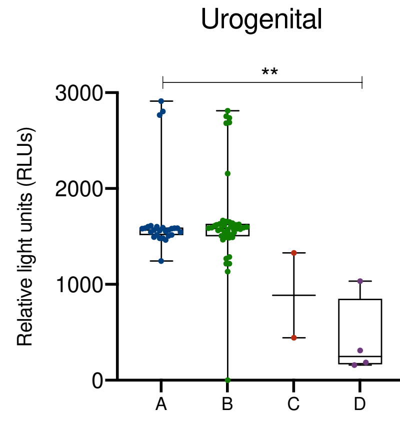

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