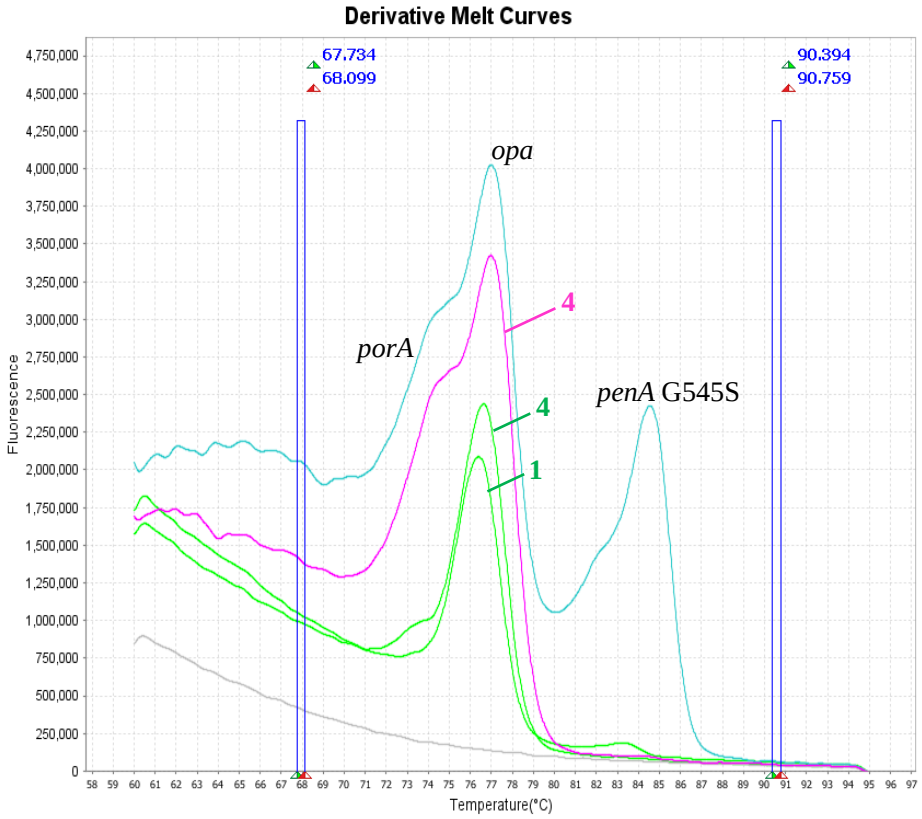
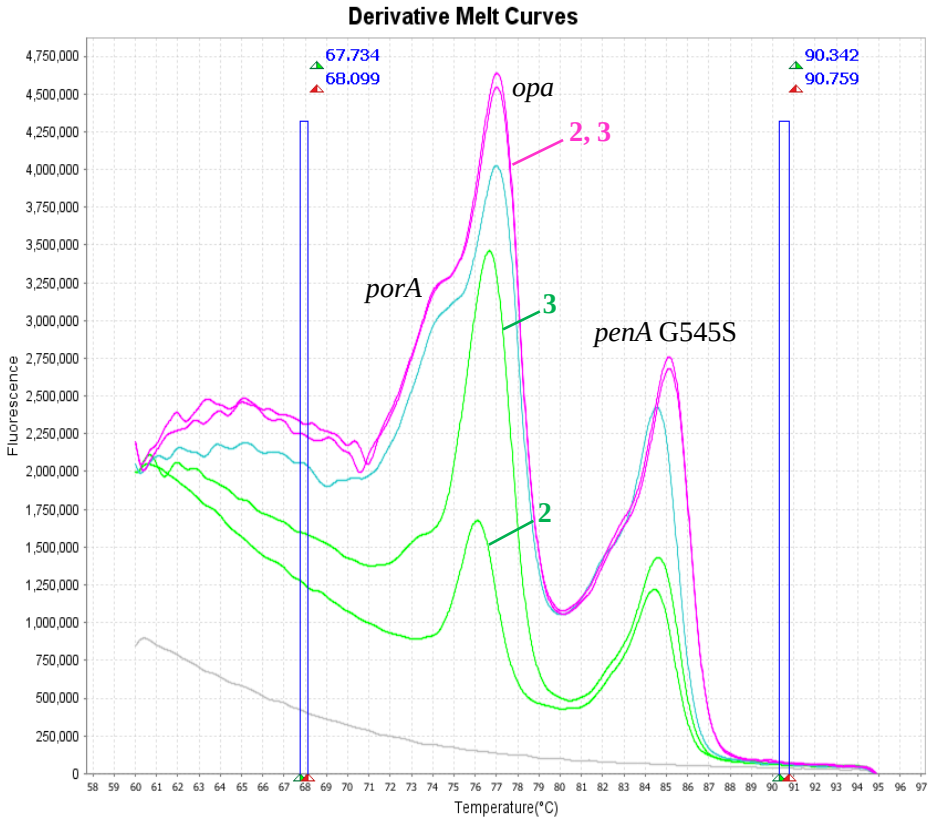
Figure S4-A: Positive pharyngeal samples

Triplex *opa* + *porA* + *penA* Gly545Ser





Light blue: control *Neisseria gonorrhoeae* (NG); mutated *penA* Gly545Ser (10^7 gDNA copies/reaction)
Pink: NG isolated from the clinical specimen (10^7 gDNA copies/reaction)
Green: pharyngeal sample
Gray: negative control

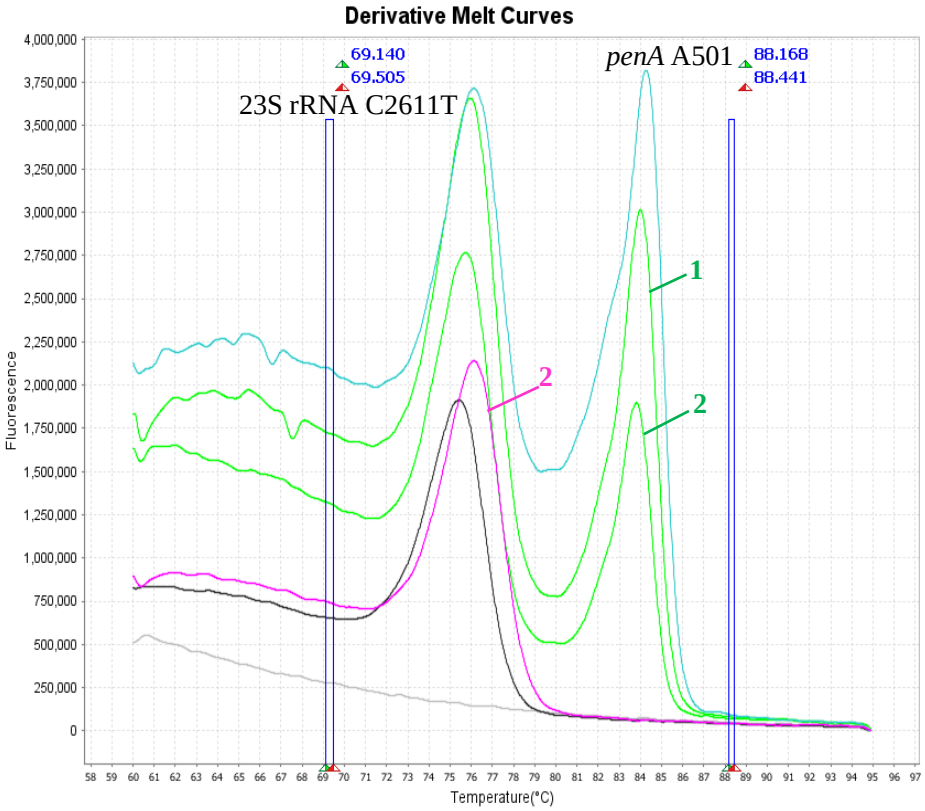
Figure S4-A. Derivative melt curves of positive pharyngeal specimens for the triplex reaction *opa* + *porA* + *penA* Gly545Ser. Light blue: control *Neisseria gonorrhoeae* (NG) with mutated *penA* Gly545Ser (10^7 gDNA copies/reaction). **Pink**: NG isolated from the clinical specimen (10^7 gDNA copies/reaction). **Green**: positive pharyngeal sample. **Gray**: negative control.

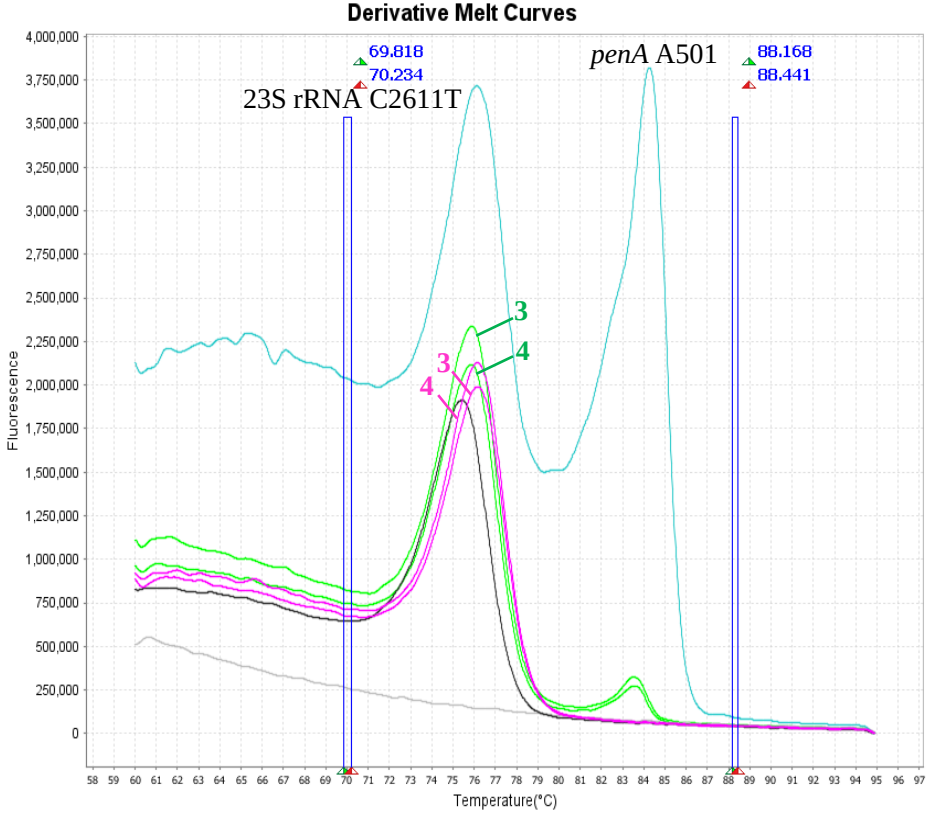
4 pharyngeal specimens positive by APTIMA CT/NG were tested. All 4 samples were positive for *opa*, whereas the *porA* reaction showed little or no amplification..

Samples 1 and 4 tested negative for the *penA* Gly545Ser reaction. Samples 2 and 3 tested positive for the *penA* Gly545Ser mutation due to low amplicon quantity. In fact, testing of gDNA extracted from the isolated culture of the same specimens showed amplification with a Tm matching wild-type *penA* Gly545.

Figure S4-B: Positive pharyngeal samples

Duplex 23S rRNA C2611T + *penA* Ala501



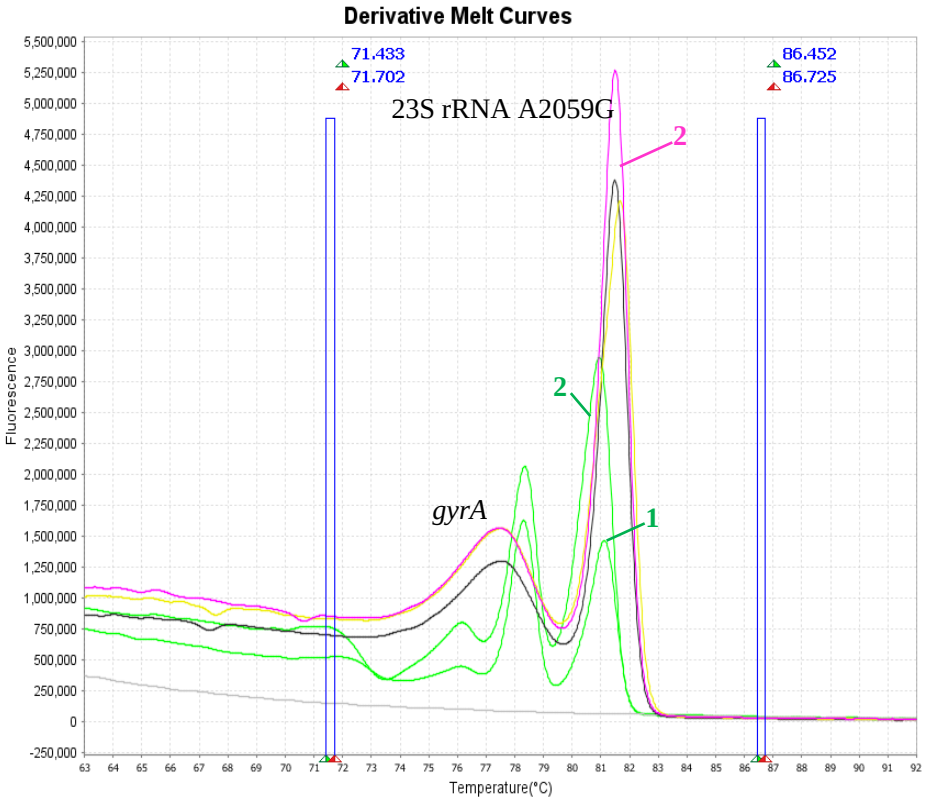


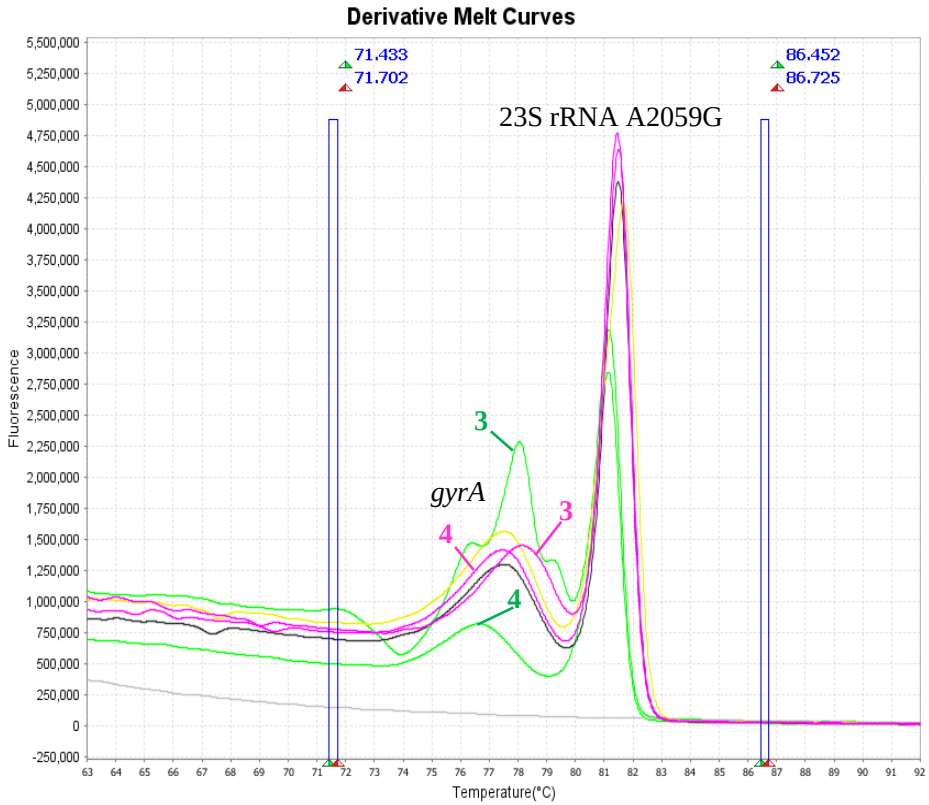
Light blue: control *Neisseria gonorrhoeae* (NG); WT 23S rRNA C2611, mosaic *penA* (10^7 gDNA copies/reaction)
Black: control NG; mutated 23S rRNA C2611T, non-mosaic *penA* (10^7 gDNA copies/reaction)
Pink: NG isolated from the clinical specimen (10^7 gDNA copies/reaction)
Green: pharyngeal sample
Gray: negative control

Figure S4-B. Derivative melt curves of positive pharyngeal specimens for the duplex reaction 23S rRNA C2611T + *penA* Ala501. **Light blue**: control *Neisseria* gonorrhoeae (NG) with wild-type (WT) 23S rRNA C2611 and mosaic *penA* (10^7 gDNA copies/reaction). **Black**: control NG with mutated 23S rRNA C2611T and non-mosaic *penA* (10^7 gDNA copies/reaction). **Pink**: NG isolated from the clinical specimen (10^7 gDNA copies/reaction). **Green**: positive pharyngeal sample. **Gray**: negative control.
4 pharyngeal specimens positive by APTIMA CT/NG were tested. All 4 clinical specimens tested WT for the 23S rRNA C2611T reaction. Clinical samples 3 and 4 showed only minor amplification of the *penA* Ala501 reaction, whereas clinical samples 1 and 2 tested positive. However, the isolated culture of sample 2 showed no amplification of the *penA* Ala501 reaction. Thus, taken together with the results of the triplex (Fig. S4 A), low bacterial load and cross-reaction with commensals may have led to erroneously assign a mosaic *penA* to sample 2.

Figure S4-C: Positive pharyngeal samples

Duplex *gyrA* Ser91Phe + 23S rRNA A2059G



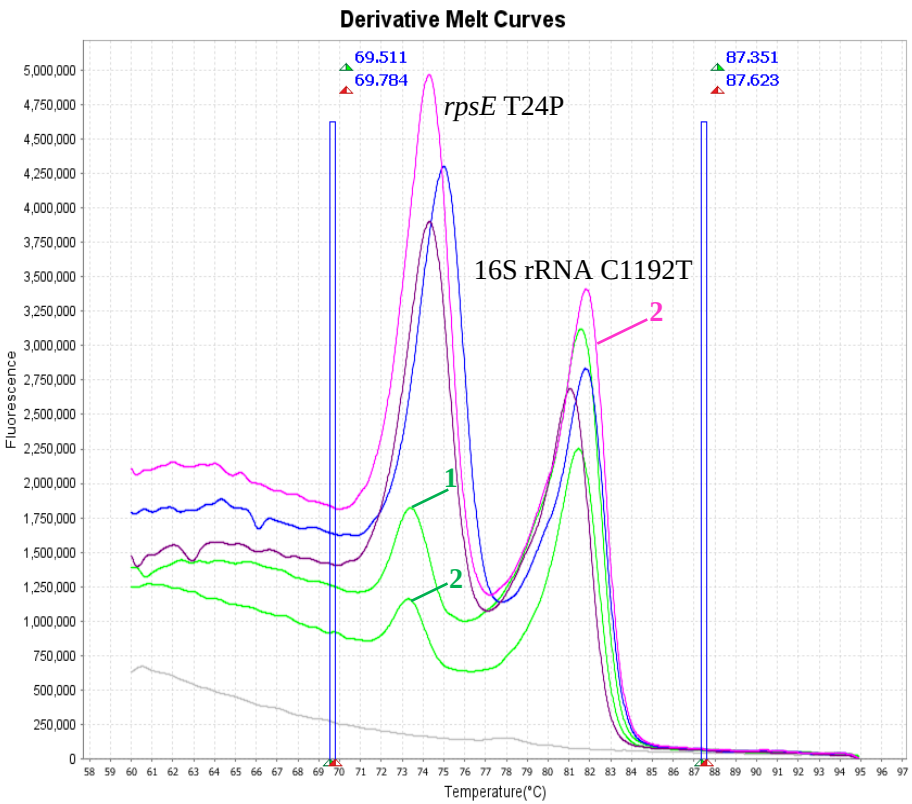


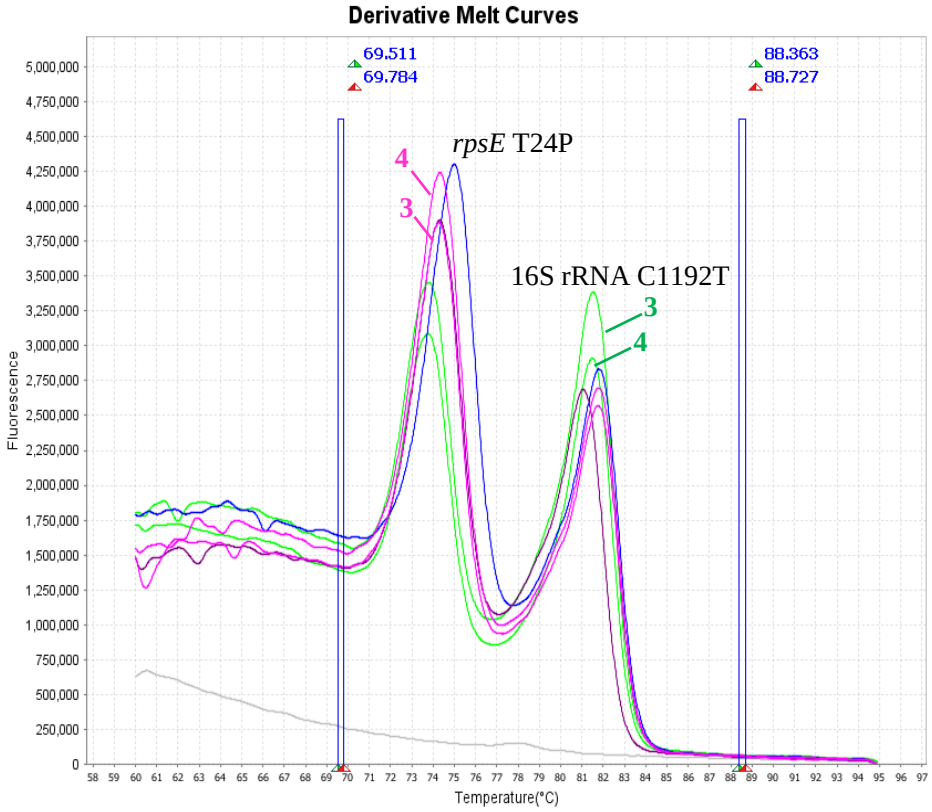
Yellow: control *Neisseria gonorrhoeae* (NG); mutated *gyrA* Ser91Phe, mutated 23S rRNA A2059G (10^7 gDNA copies/reaction)
Black: control NG; mutated *gyrA* Ser91Phe, WT 23S rRNA A2059 (10^7 gDNA copies/reaction)
Pink: isolated culture from the specimen (10^7 gDNA copies/reaction)
Green: pharyngeal sample
Gray: negative control

Figure S4-C. Derivative melt curves of positive pharyngeal specimens for the duplex reaction *gyrA* Ser91Phe + 23S rRNA A2059G. **Yellow**: control *Neisseria gonorrhoeae* (NG) with mutated *gyrA* Ser91Phe and mutated 23S rRNA A2059G (10^7 gDNA copies/reaction). **Black**: control NG with mutated *gyrA* Ser91Phe and wild-type (WT) 23S rRNA A2059 (10^7 gDNA copies/reaction)
Pink: NG isolated from the clinical specimen (10^7 gDNA copies/reaction). **Green**: positive pharyngeal sample. **Gray**: negative control.
4 pharyngeal specimens positive by APTIMA CT/NG were tested. Both *gyrA* Ser91Phe and 23S rRNA A2059G reactions were not interpretable for all 4 specimens tested. All 3 isolated cultures exhibited WT 23S rRNA A2059. The isolated culture from specimen 3 exhibited a WT GyrA, whereas the isolated culture from specimens 2 and 4 exhibited a GyrA Ser91Phe substitution.

Figure S4-D: Positive pharyngeal samples

Duplex *rpsE* Thr24Pro + 16S rRNA C1192T



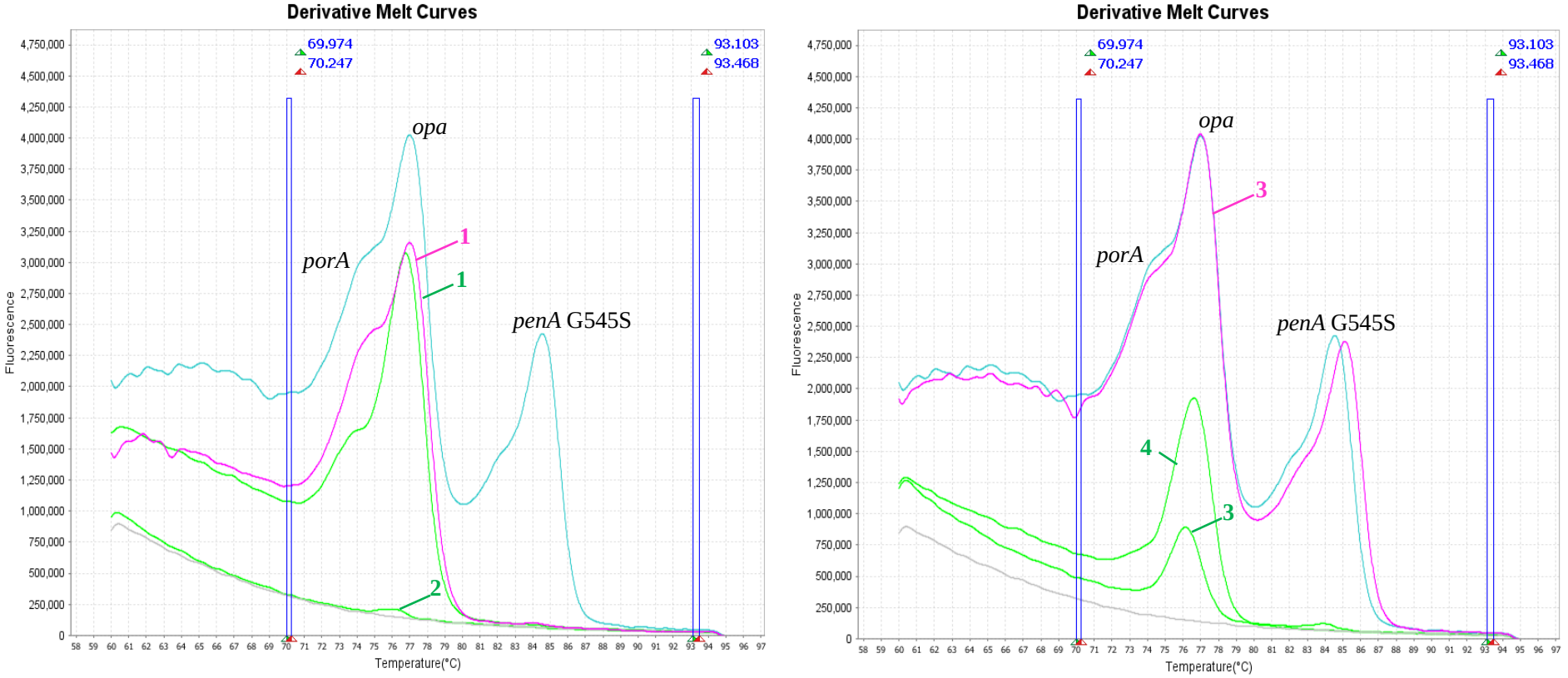


Purple: control *Neisseria gonorrhoeae* (NG); WT *rpsE* Thr24, mutated 16S rRNA C1192T (10^7 gDNA copies/reaction)
Blue: control NG; mutated *rpsE* Thr24Pro, WT 16S rRNA C1192 (10^7 gDNA copies/reaction)
Pink: isolated culture from the specimen (10^7 gDNA copies/reaction)
Green: pharyngeal sample
Gray: negative control

Figure S4-D. Derivative melt curves of positive pharyngeal specimens for the duplex reaction *rpsE* Thr24Pro + 16S rRNA C1192T. **Purple:** control *Neisseria gonorrhoeae* (NG) with wild-type (WT) *rpsE* Thr24 and mutated 16S rRNA C1192T (10^7 gDNA copies/reaction). **Blue**: control NG with mutated *rpsE* Thr24Pro and WT 16S rRNA C1192(10^7 gDNA copies/reaction). **Pink**: NG isolated from the clinical specimen (10^7 gDNA copies/reaction). **Green**: positive pharyngeal sample. **Gray**: negative control.
4 pharyngeal specimens positive by APTIMA CT/NG were tested. In sample 1 and 2 amplfication of reaction *rpsE* Thr24Pro was very poor. In samples 3 and 4 a strong Tm decrease compared to the WT control was observed. The 16S rRNA C1192T reaction yielded good amplicon amounts with a Tm indicative for the absence of the resistance mutation in all 4 tested samples.

Figure S5-A: Positive rectal samples

Triplex *opa* + *porA* + *penA* Gly545Ser

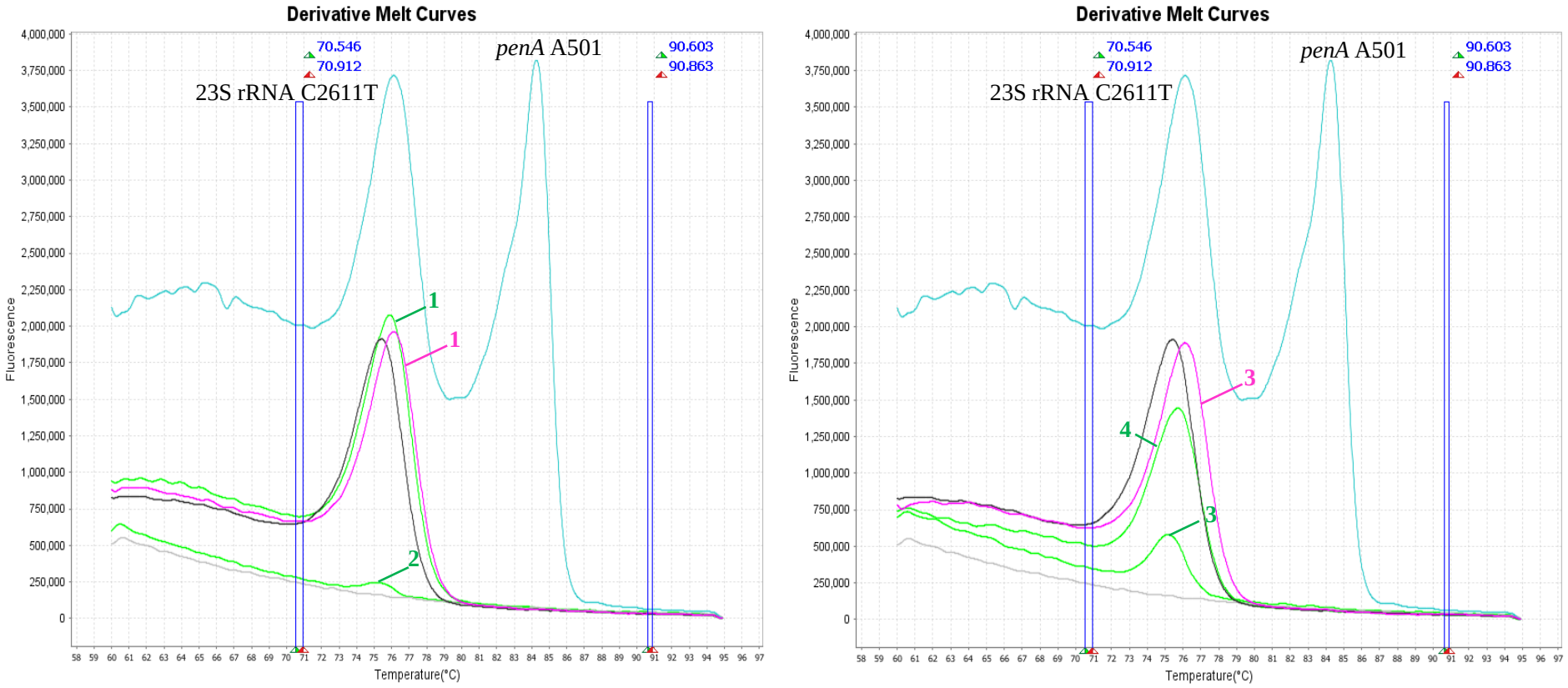


Light blue: control *Neisseria gonorrhoeae* (NG); mutated *penA* Gly545Ser (10^7 gDNA copies/reaction)
Pink: isolated culture from the specimen (10^7 gDNA copies/reaction)
Green: rectal sample
Gray: negative control

Figure S5-A. Derivative melt curves of positive rectal specimens for the triplex reaction *opa* + *porA* + *penA* Gly545Ser. Light blue: control *Neisseria gonorrhoeae* (NG) with mutated *penA* Gly545Ser (10^7 gDNA copies/reaction). **Pink**: NG isolated from the clinical specimen (10^7 gDNA copies/reaction). **Green**: positive rectal sample. **Gray**: negative control.
4 rectal specimens positive by APTIMA CT/NG were tested. Sample 1 was positive for both detection genes *opa* and *porA*, whereas sample 3 and 4 were positive only for the *opa* reaction. Sample 2 tested negative for both reactions. All samples tested negative for the *penA* Gly545Ser reaction. Testing of the culture isolated from specimen 3 showed amplification with a Tm matching wild-type *penA* Gly545.

Figure S5-B: Positive rectal samples

Duplex 23S rRNA C2611T + *penA* Ala501

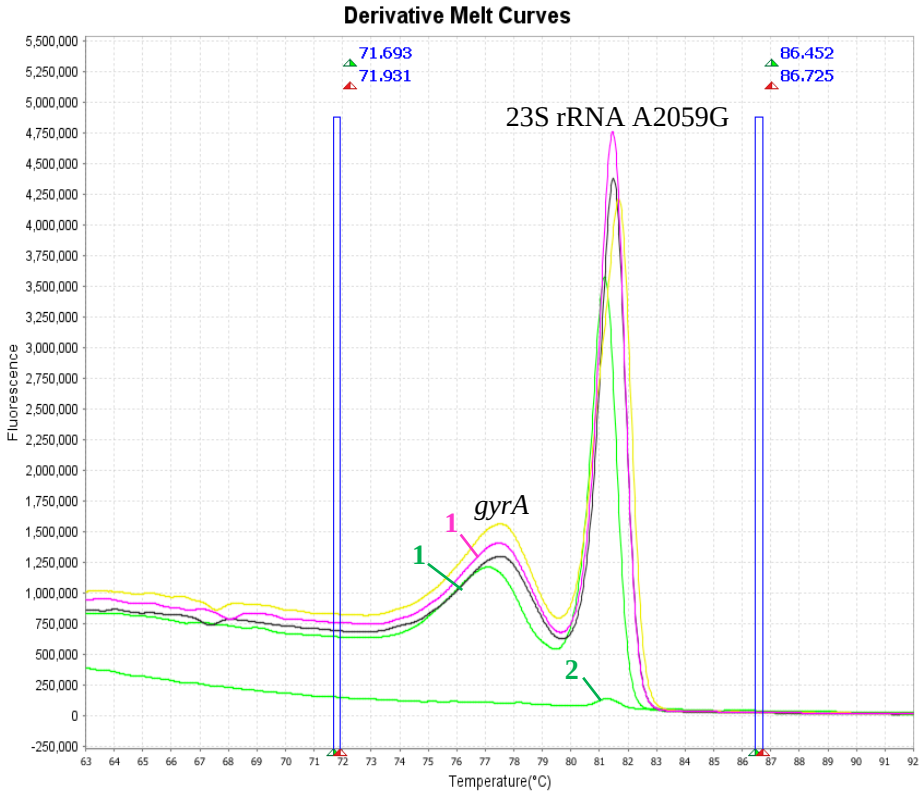


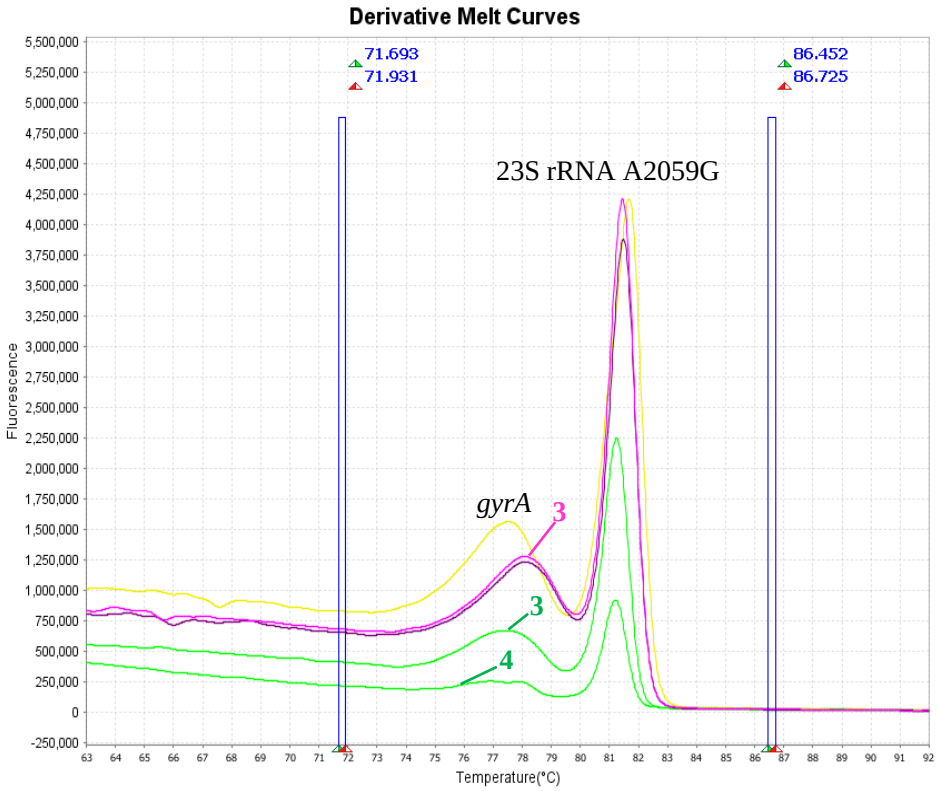
Light blue: control *Neisseria gonorrhoeae* (NG); WT 23S rRNA C2611, mosaic *penA* (10^7 gDNA copies/reaction)
Black: control NG; mutated 23S rRNA C2611T, non-mosaic *penA* (10^7 gDNA copies/reaction)
Pink: isolated culture from the specimen (10^7 gDNA copies/reaction)
Green: rectal sample
Gray: negative control

Figure S5-B. Derivative melt curves of positive rectal specimens for the duplex reaction 23S rRNA C2611T + *penA* Ala501. **Light blue**: control *Neisseria gonorrhoeae* (NG) with wild-type (WT) 23S rRNA C2611 and mosaic *penA* (10^7 gDNA copies/reaction). **Black**: control NG with mutated 23S rRNA C2611T and non-mosaic *penA* (10^7 gDNA copies/reaction). **Pink**: NG isolated from the clinical specimen (10^7 gDNA copies/reaction). **Green**: positive rectal sample. **Gray**: negative control.
4 rectal specimens positive by APTIMA CT/NG were tested. Samples 1 and 4 showed a Tm in the range for WT 23S rRNA C2611, whereas samples 2 and 3 could not be interpreted due to low amplicon quantity. The Tm of specimen 3 falls in the range expected for mutated 23S rRNA C2611T. However, testing of the isolated culture of specimen 3 clearly showed a Tm matching WT 23S rRNA C2611. No sample tested positive for the *penA* Ala501 reaction.

Figure S5-C: Positive rectal samples

Duplex *gyrA* Ser91Phe + 23S rRNA A2059G



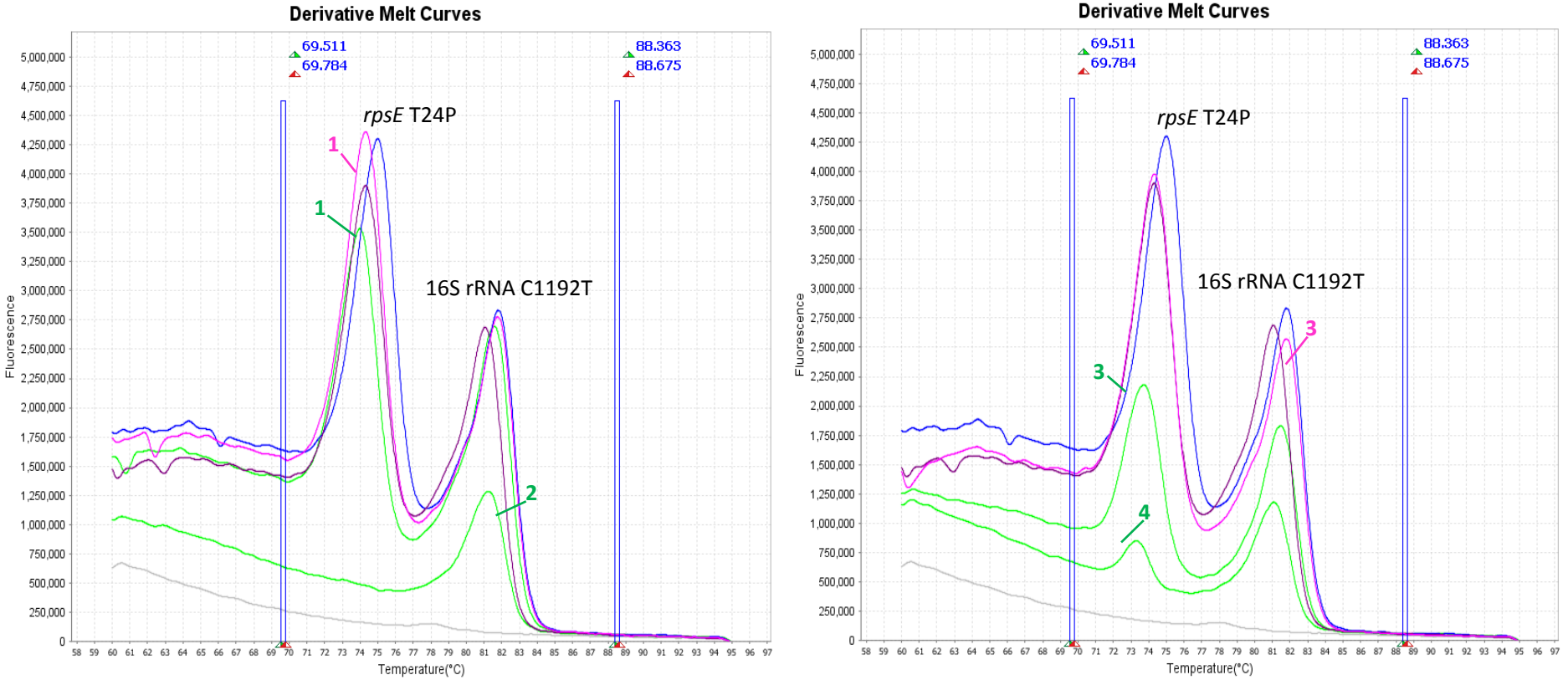


Yellow: control NG; mutated *gyrA* Ser91Phe, mutated 23S rRNA A2059G (10^7 gDNA copies/reaction)
Black: control NG; mutated *gyrA* Ser91Phe, WT 23S rRNA A2059 (10^7 gDNA copies/reaction)
Purple: control NG; WT *gyrA* Ser91Phe, WT 23S rRNA A2059 (10^7 gDNA copies/reaction)
Pink: isolated culture from the specimen (10^7 gDNA copies/reaction)
Green: rectal sample
Gray: negative control

Figure S5-C. Derivative melt curves of positive rectal specimens for the duplex reaction *gyrA* Ser91Phe + 23S rRNA A2059G. **Yellow**: control *Neisseria gonorrhoeae* (NG) with mutated *gyrA* Ser91Phe and mutated 23S rRNA A2059G (10^7 gDNA copies/reaction). **Black**: control NG with mutated *gyrA* Ser91Phe and wild-type (WT) 23S rRNA A2059 (10^7 gDNA copies/reaction)
Pink: NG isolated from the clinical specimen (10^7 gDNA copies/reaction). **Green**: positive rectal sample. **Gray**: negative control.
4 rectal specimens positive by APTIMA CT/NG were tested. The 23S rRNA A2059G reaction was not interpretable for all 4 specimens. Both cultures isolated from specimens 1 and 3 exhibited WT 23S rRNA A2059. The *gyrA* Ser91Phe reaction for specimen 1 showed an amplicon with a Tm in the range expected for the mutated *gyrA*. This result was confirmed by testing of the culture isolated from the specimen. The *gyrA* Ser91Phe reaction for specimen 3 showed an amplicon with a Tm in the range expected for the mutated *gyrA* which was due to low amplicon quantity. In fact, testing of the culture isolated from specimen 3 showed the presence of WT GyrA. There was no amplification of the *gyrA* Ser91Phe reaction for specimens 2 and 4.

Figure S5-D: Positive rectal samples

Duplex *rpsE* Thr24Pro + 16S rRNA C1192T

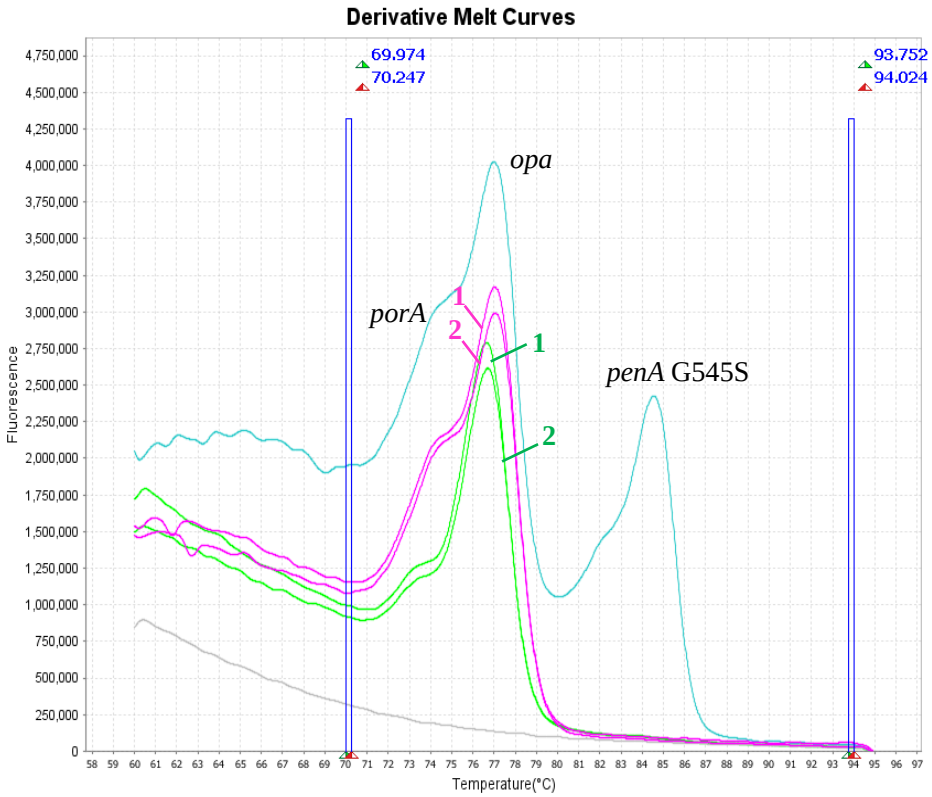


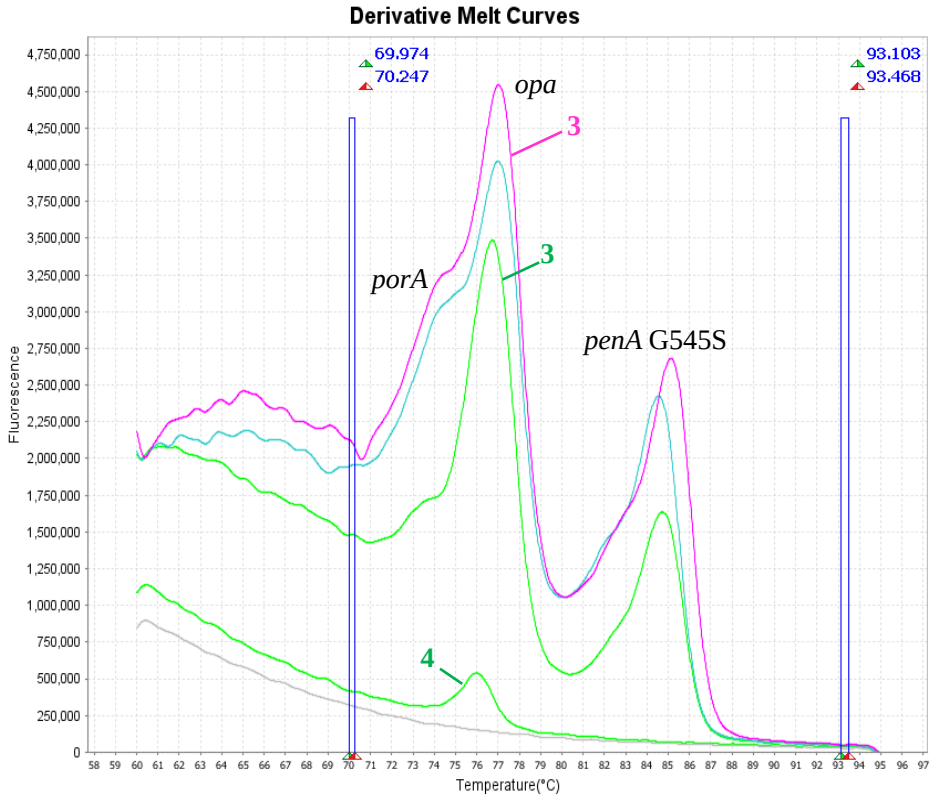
Purple: control NG; WT *rpsE* Thr24, mutated 16S rRNA C1192T (10^7 gDNA copies/reaction)
Blue: control NG; mutated *rpsE* Thr91Pro, WT 16S rRNA C1192 (10^7 gDNA copies/reaction)
Pink: isolated culture from the specimen (10^7 gDNA copies/reaction)
Green: rectal sample
Gray: negative control

Figure S5-D. Derivative melt curves of positive rectal specimens for the duplex reaction *rpsE* Thr24Pro + 16S rRNA C1192T. **Purple:** control *Neisseria gonorrhoeae* (NG) with wild-type (WT) *rpsE* Thr24 and mutated 16S rRNA C1192T (10^7 gDNA copies/reaction). **Blue**: control NG with mutated *rpsE* Thr91Pro and WT 16S rRNA C1192 (10^7 gDNA copies/reaction). **Pink**: NG isolated from the clinical specimen (10^7 gDNA copies/reaction). **Green**: positive rectal sample. **Gray**: negative control.
4 rectal specimens positive by APTIMA CT/NG were tested. The 16S rRNA C1192T reaction was amplified in all 4 samples, although a decrease of the Tm due to low amplicon quantity was observed, which would result in false positives when comparing to the Tm of the controls. The *rpsE* Thr24Pro reaction was amplified only in samples 1 and 3 showing again a strong decrease of Tm compared to the controls.

Figure S6-A: Positive urethral samples

Triplex *opa* + *porA* + *penA* Gly545Ser



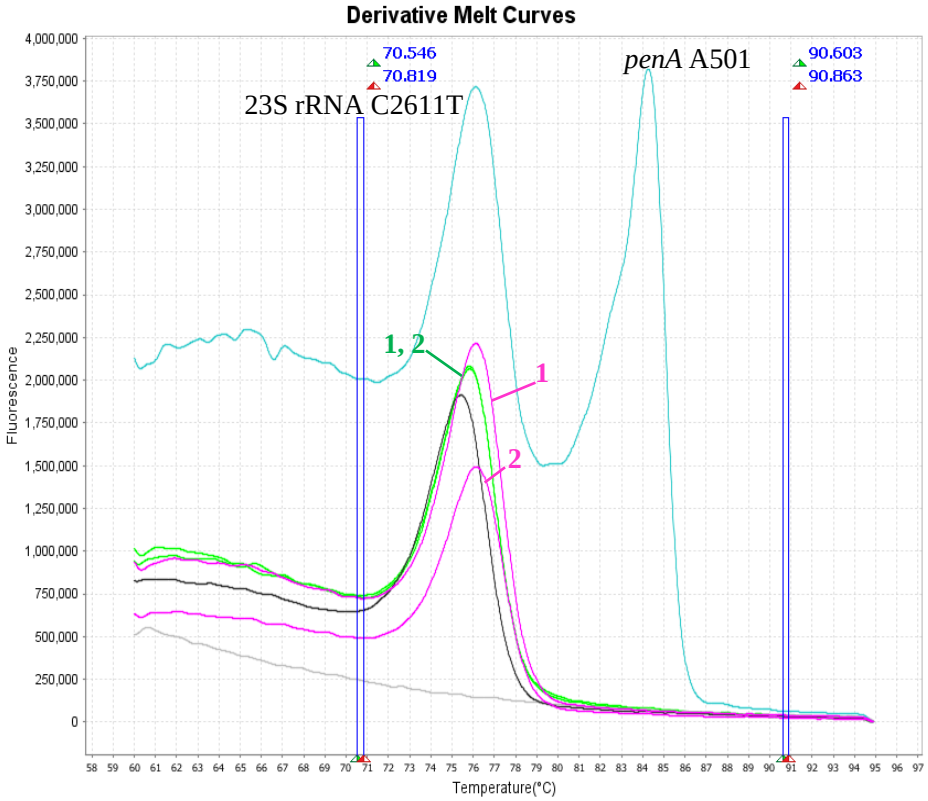


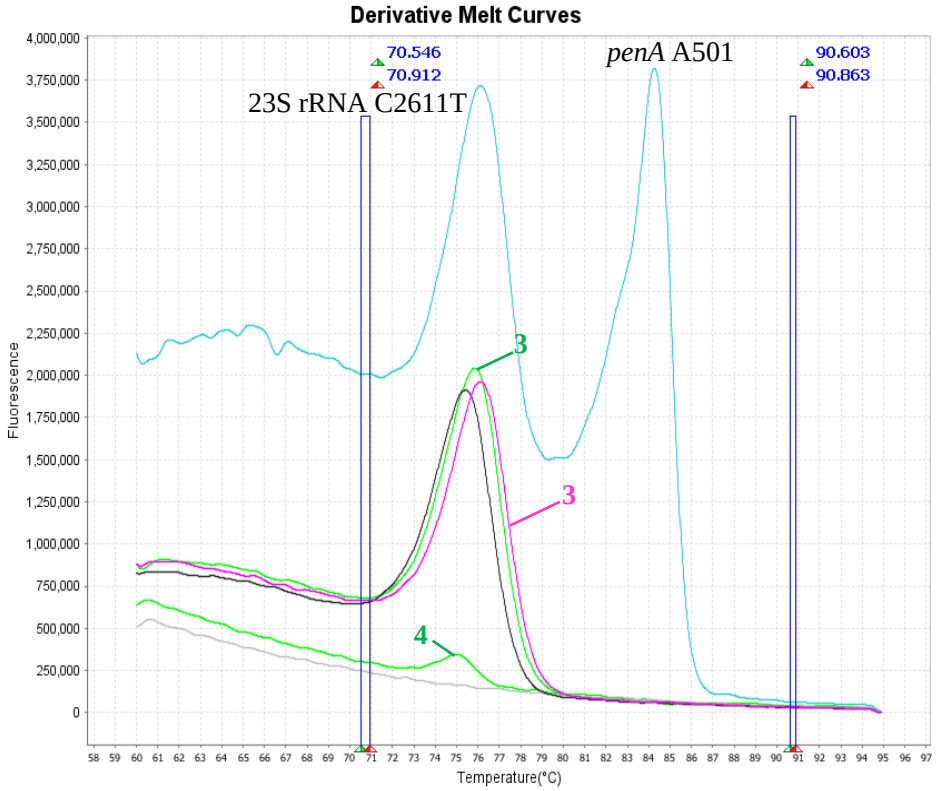
Light blue: control NG; mutated *penA* Gly545Ser (10^7 gDNA copies/reaction)
Pink: isolated culture from the specimen (10^7 gDNA copies/reaction)
Green: urethral sample
Gray: negative control

Figure S6-A. Derivative melt curves of positive urethral specimens for the triplex reaction *opa* + *porA* + *penA* Gly545Ser. Light blue: control *Neisseria gonorrhoeae* (NG) with mutated *penA* Gly545Ser (10^7 gDNA copies/reaction). **Pink**: NG isolated from the clinical specimen (10^7 gDNA copies/reaction). **Green**: positive urethral sample. **Gray**: negative control. 4 urethral specimens positive by APTIMA CT/NG were tested. 3 samples were positive for both detection genes *opa* and *porA*, whereas sample 4 showed only low amplification of the *opa* reaction. Samples 1 and 2 tested negative for the *penA* Gly545Ser reaction. Sample 3 tested positive for the *penA* Gly545Ser mutatedation. However, testing of gDNA extracted from the isolated culture of the same specimen clearly showed amplification with a Tm matching wild-type *penA* Gly545.

Figure S6-B: Positive urethral samples

Duplex 23S rRNA C2611T + *penA* Ala501



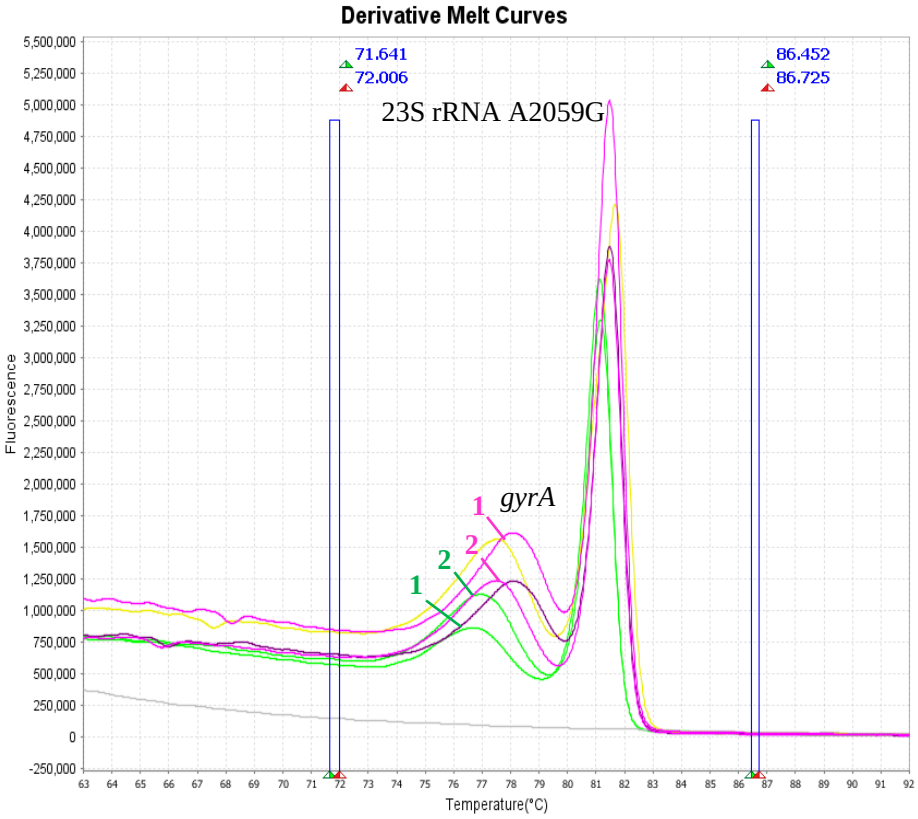


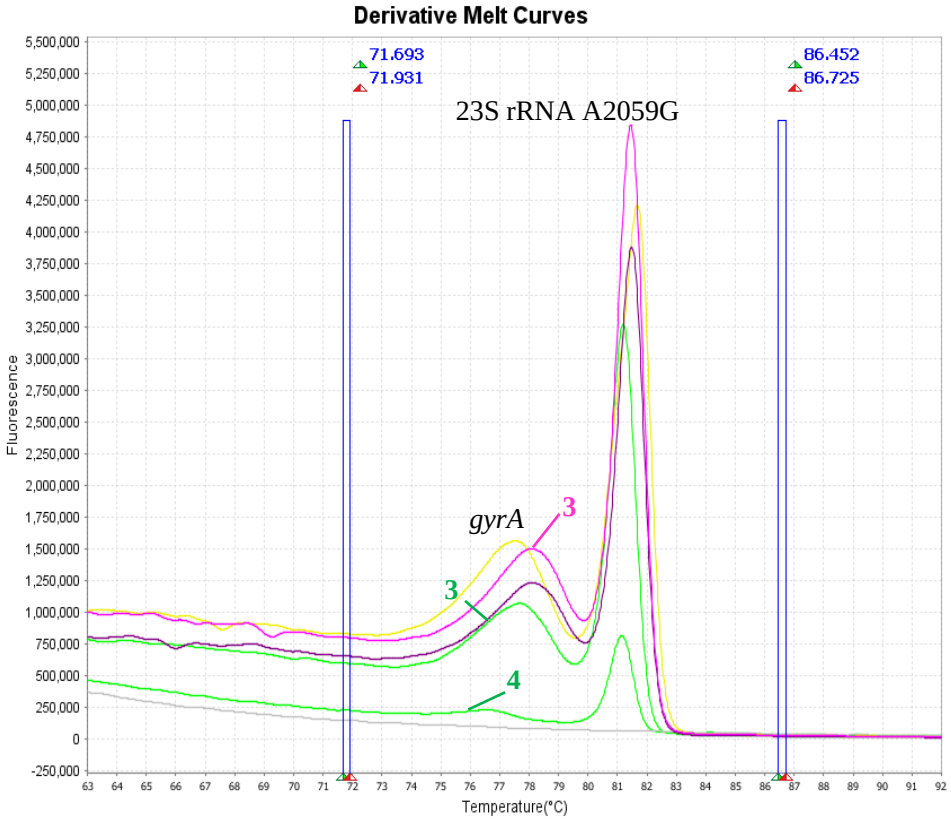
Light blue: control NG; WT 23S rRNA C2611, mosaic *penA* XXXIV (10^7 gDNA copies/reaction)
Black: control NG; mutated 23S rRNA C2611T, non-mosaic *penA* (10^7 gDNA copies/reaction)
Pink: isolated culture from the specimen (10^7 gDNA copies/reaction)
Green: urethral sample
Gray: negative control

Figure S6-B. Derivative melt curves of positive urethral specimens for the duplex reaction 23S rRNA C2611T + *penA* Ala501. **Light blue**: control *Neisseria gonorrhoeae* (NG) with wild-type (WT) 23S rRNA C2611 and mosaic *penA* (10^7 gDNA copies/reaction). **Black**: control NG with mutated 23S rRNA C2611T and non-mosaic *penA* (10^7 gDNA copies/reaction). **Pink**: NG isolated from the clinical specimen (10^7 gDNA copies/reaction). **Green**: positive urethral sample. **Gray**: negative control.
4 urethral specimens positive by APTIMA CT/NG were tested. 3 samples exhibited WT 23S rRNA C2611, whereas sample 4 showed very low amplification for this reaction. No sample tested positive for the *penA* Ala501 reaction.

Figure S6-C: Positive urethral samples

Duplex *gyrA* Ser91Phe + 23S rRNA A2059G



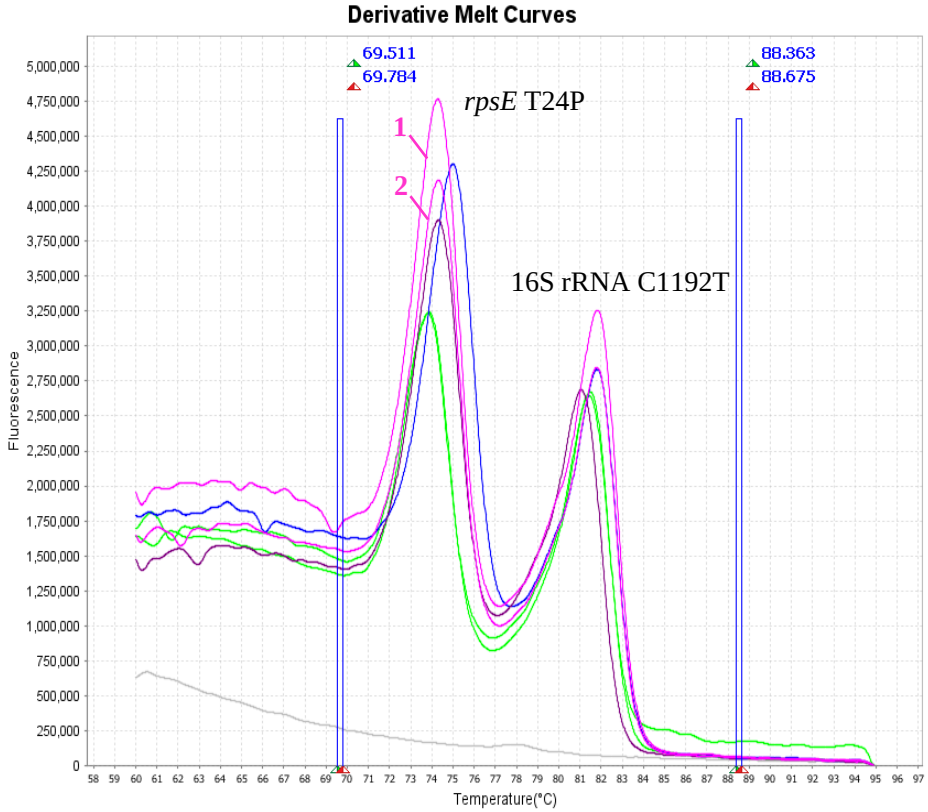


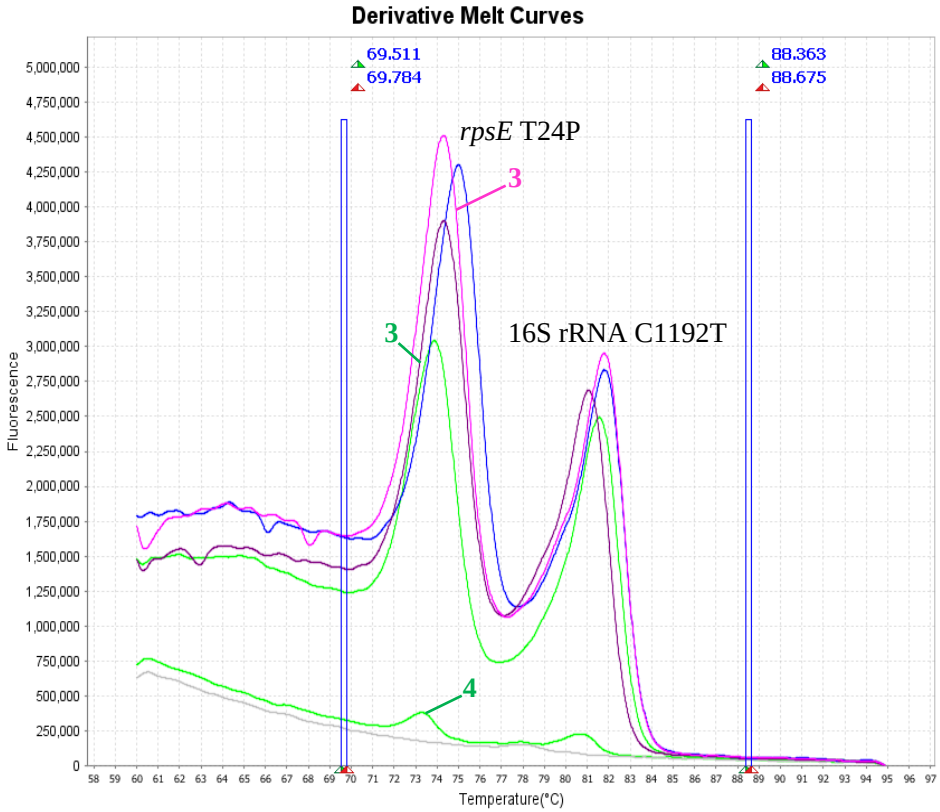
Yellow: control NG; mutated *gyrA* Ser91Phe, mutated 23S rRNA A2059G (10^7 gDNA copies/reaction)
Purple: control NG; WT *gyrA* Ser91Phe, WT 23S rRNA A2059 (10^7 gDNA copies/reaction)
Pink: isolated culture from the specimen (10^7 gDNA copies/reaction)
Green: urethral sample
Gray: negative control

Figure S6-C. Derivative melt curves of positive urethral specimens for the duplex reaction *gyrA* Ser91Phe + 23S rRNA A2059G. **Yellow**: control *Neisseria gonorrhoeae* (NG) with mutated *gyrA* Ser91Phe and mutated 23S rRNA A2059G (10^7 gDNA copies/reaction). **Black**: control NG with mutated *gyrA* Ser91Phe and wild-type (WT) 23S rRNA A2059 (10^7 gDNA copies/reaction)
Pink: NG isolated from the clinical specimen (10^7 gDNA copies/reaction). **Green**: positive urethral sample. **Gray**: negative control.
4 urethral specimens positive by APTIMA CT/NG were tested. The 23S rRNA A2059G reaction was not interpretable for all 4 specimens tested. All 3 isolated cultures exhibited WT 23S rRNA A2059. The *gyrA* Ser91Phe reaction for specimen 1 and 2 was not interpretable due to low amplicon quantities. The isolated cultures from samples 1 and 2 exhibited a WT and a mutated *gyrA*, respectively. The *gyrA* Ser91Phe reaction for specimen 3 showed a Tm in the range expected for the mutated *gyrA* due to low amplicon quantity. In fact, testing of the culture isolated from the specimen showed the presence of WT *gyrA*. There was little amplification of both reactions for specimen 4.

Figure S6-D: Positive urethral samples

Duplex *rpsE* Thr24Pro + 16S rRNA C1192T





Purple: control NG; WT *rpsE* Thr24, mutated 16S rRNA C1192T (10^7 gDNA copies/reaction)
Blue: control NG; mutated *rpsE* Thr91Pro, WT 16S rRNA C1192 (10^7 gDNA copies/reaction)
Pink: isolated culture from the specimen (10^7 gDNA copies/reaction)
Green: urethral sample
Gray: negative control

Figure S6-D. Derivative melt curves of positive urethral specimens for the duplex reaction *rpsE* Thr24Pro + 16S rRNA C1192T. **Purple:** control *Neisseria gonorrhoeae* (NG) with wild-type (WT) *rpsE* Thr24 and mutated 16S rRNA C1192T (10^7 gDNA copies/reaction). **Blue**: control NG with mutated *rpsE* Thr91Pro and WT 16S rRNA C1192 (10^7 gDNA copies/reaction). **Pink**: NG isolated from the clinical specimen (10^7 gDNA copies/reaction). **Green**: positive urethral sample. **Gray**: negative control.
4 urethral specimens positive by APTIMA CT/NG were tested. In samples 1, 2 and 3 both reactions yielded a good amplification, although a decrease of the amplicon Tm compared to the WT control was observed. All 3 samples tested negative for both mutatedations. There was very low amplification for both reactions in sample 4.