

Evaluation of new HyBeacon-based PCR assays for the direct detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

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Introduction and Purpose

We evaluated the performance of the new FluoroType® (FT) CT and FluoroType® NG assays (Hain Lifescience, Nehren, Germany) for the direct detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) in genitourinary specimens. Results were compared to already established PCR assays and to conventional culture for NG.

CT-Specimens

• Comparison of FT CT with GenoQuick® CT (Hain Lifescience): 104 urines, 89 cervical swabs, 9 ejaculates [Results: Table 1, 2, 3].

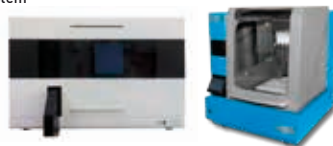
NG-Specimens

• Comparison of FT NG versus NG-culture: 46 swabs and urine samples [Results: Table 4, 6].
• Comparison of FT NG versus inhouse-PCR [Whiley et al., Eur J Clin Microbiol Infect Dis (2004) 23: 705-10], LightCycler® 2.0 (Roche, Mannheim, Germany): 36 DNA samples from swabs and urines, DNA isolates [Laboratory Volkmann, Karlsruhe, Germany] [Results: Table 5, 6].

Methods

DNA purification: Fully automated GenoXtract® System (Hain Lifescience) (see Figure 1) and manual FluoroLyse extraction kit (Hain Lifescience).

Fig. 1: GenoXtract® System



PCR: The new FT NG and CT tests are based on HyBeacon probe technology. HyBeacon probes are dye-labeled, single-stranded and self-quenching oligonucleotides, which emit light when hybridized to a DNA target. Evaluation of the DNA structure is done by a melting curve evaluation on the FluoroCycler® (see Figures 2, 3, 4, 5).

Culture for NG: Thayer-Martin, Chocolate and Columbia blood agar.

Fig. 2: FluoroCycler® – HyBeacon Probes

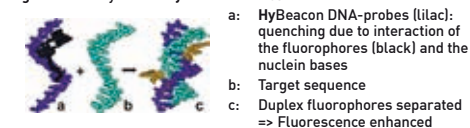


Fig. 3: FluoroCycler® – Setup

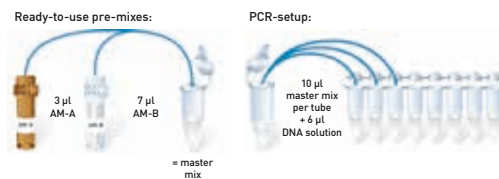
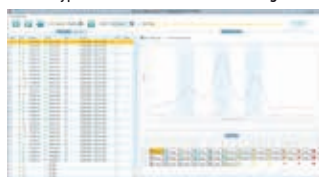


Fig. 4: FluoroCycler® – Amplification and detection: 2 h 15 min



Fig. 5: FluoroType® CT and FluoroType® NG – Detection with Melting Curves

CT-specific melting points (T_m) are 61.0 °C and 72.3 °C. The Internal Control DNA (IC) has a melting point of 48.0 °C.



Results

Table 1: Results of FluoroType® CT with FluoroLyse DNA isolation compared to GenoQuick® CT*.

FluoroType® CT with FluoroLyse, n=202	GenoQuick® CT	
	+	-
+	85	1
-	0	116

* Results correspond to those obtained after repeated testing of false-positive specimens (7), false-negative specimens (2) or inhibited specimens (1) with the FluoroType® CT and GenoQuick® CT assay.

Table 2: Results of FluoroType® CT with GenoXtract® DNA isolation compared to GenoQuick® CT*.

FluoroType® CT with GenoXtract®, n=201**	GenoQuick® CT	
	+	-
+	89	3
-	0	109

* Results correspond to those obtained after repeated testing of false-positive specimens (14), false-negative specimens (2) or inhibited specimens (2 **) with the FluoroType® CT and GenoQuick® CT assay.

** 1 of the 202 specimen was inhibited in the FluoroType® CT test but not in the GenoQuick® CT also after repeated testing. It was therefore excluded from the calculations.

Table 3: Summarized results of the CT study.

	Sensitivity [%]	Specificity [%]	NPV [%]	PPV [%]
FluoroType® CT with FluoroLyse	100.0	99.14	100.0	98.83
FluoroType® CT with GenoXtract®	100.0	97.32	100.0	96.74

Table 4: Results of FluoroType® NG compared to NG culture.

FluoroType® NG, n=46	NG Culture	
	+	-
+	20	3*
-	0	23

* Positive PCR-result confirmed by a reference NG-PCR (Laboratory Volkmann, Karlsruhe, Germany).

Table 5: Results of FluoroType® NG compared to a reference PCR-method*.

FluoroType® NG, n=36	Reference NG-PCR (LightCycler®)	
	+	-
+	21	0
-	0	15

* Whiley et al., Eur J Clin Microbiol Infect Dis (2004) 23: 705-10.

Table 6: Summarized results of the NG study.

	Sensitivity [%]	Specificity [%]
FluoroType® NG versus culture and PCR	100.0	100.0
FluoroType® CT versus inhouse PCR	100.0	100.0

Conclusions

Both PCR-assays, the FluoroType® CT and FluoroType® NG for the direct detection of *C. trachomatis* and *N. gonorrhoeae* provide sensitive and specific results in about 3 hours.