P-1918

Evaluation of the new HyBeacon-based PCR assay, FluoroType MTB, for the direct detection of *M. tuberculosis* in respiratory and non-respiratory specimens

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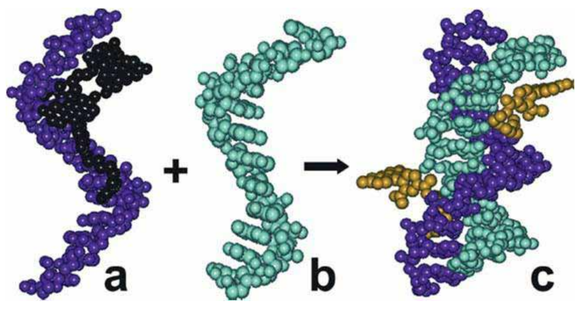
Objectives

The performance of the new **Fluoro**Type (FT) **MTB** assay (Hain Lifescience, Nehren, Germany) for the direct detection of *Mycobacterium tuberculosis* complex in respiratory and nonrespiratory specimens was evaluated. Results were compared to conventional liquid and solid culture media. In addition a subgroup of the specimens were also tested with the Cobas TaqMan (CT) MTB test (Roche, Mannheim, Germany).

Methods

In total 281, 2% N-acetyl-L-cysteine/ sodium hydroxide (NALC)-decontaminated specimens were investigated with the FT MTB assay. Microscopy was performed directly from the patient specimens (except urines, n= 20). After decontamination 500µl of the phosphate-buffered suspension was taken for inoculation of BACTEC MGIT medium and 100µl each for solid media (Löwenstein-Jensen, Stonebrink). For DNA extraction 700µl of the suspension was incubated at 70°C for 15min. DNA purification was performed fully automated in the **Geno**Xtract and with the GXT DNA/RNA extraction kit (Hain Lifescience) in 40min. Identification of cultured acid fast bacteria was performed with the **Geno**Type **MTBC** and **Geno**Type **Mycobacteria CM/AS** strip assays (Hain Lifescience). The new FT MTB test is based on **Hy**Beacon fluorescence-technology and is performed in the **Fluoro**Cycler (Hain Lifescience) (see Fig. 1). After PCR amplification melting curves are created with **Hy**Beacon probes at probe specific temperatures (see Fig. 2). The Cobas TaqMan MTB test is based on real-time PCR technology and is recommended for the use of respiratory specimens. DNA preparation is done manually with an aliquot of 100µl liquefied, decontaminated and concentrated specimen which is transferred to 500µl washing buffer. After a centrifugation step, 100µl lysis buffer is added. After incubation at 65°C for 45min, 100µl neutralization buffer is added and 50µl of the DNA-lysate are used for PCR. The FT MTB and the CT MTB assay were performed according to the manufacturer´s instructions.

Fig. 1 **Hy**Beacon probes

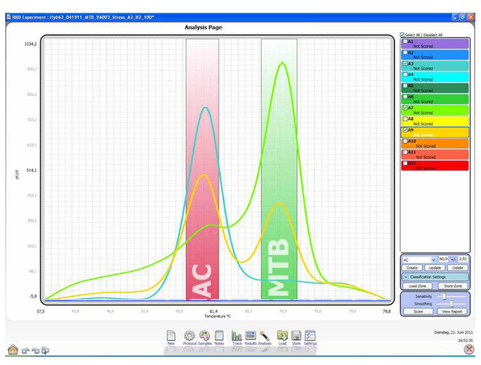


a: **Hy**Beacon DNA-probes
Quenching due to interaction of the fluorophores

b: Target sequence

c: Duplex
Fluorophores separated => Fluorescence enhanced

Fig. 2 **Fluoro**Type **MTB** – Detection with melting curves



Melting temperature of the MTB-sequence specific amplicon: 71°C (+/- 3°C)

Melting temperature of the of the amplification control amplicon: 59.5°C (+/- 3°C)

Results

280 respiratory and non-respiratory specimens were utilized for **Fluoro**Type **MTB** test. 79 of 280 specimens were culture-positive (incl. clinic) for *M. tuberculosis*. FT MTB correctly identified 48 of 48 smear-positive, 30 of 31 smear-negative specimens. One sputum specimen with positive culture (microscopic-negative) was negative with the FT MTB assay. In 201 culture-negative specimens, FT MTB showed 199 correct negative results. Performance of the FT MTB assay for respiratory and non-respiratory specimens is shown in Tables 1-3. 77 respiratory specimens were utilized for CT MTB assay, 39 were culture-positive (incl. clinic) for *M. tuberculosis*. CT MTB correctly identified 13 of 13 smear-positive, 25 of 26 smear-negative specimens. In 42 culture-negative specimens, CT MTB showed 42 correct negative results. Performance results of the Cobas TaqMan test are shown in Tables 4, 5. Overall sensitivity, specificity, NPV and PPV were 98.7%, 99.0%, 99.5% and 97.5% with the FT MTB assay and 97.4%, 100%, 97.4% and 100% with the CT MTB-PCR, respectively. Overall sensitivity in smear-negative specimens was 96.7% with the FT MTB test and 96.1% with the CT MTB test.

Table 1. Results FT MTB-PCR Respiratory specimens (n= 255)

n= 51 specimens: microscopic positive/scanty

Culture/Clinic	FT MTB	n	Performance
pos	pos	47	RP
pos	neg	0	FN
neg	neg	4	RN

Parameter	FT MTB
Sens (%)	100
Spec (%)	100

Table 2. Results FT MTB-PCR Respiratory specimens (n= 255)

n= 204 specimens: microscopic negative

Culture/Clinic	FT MTB	n	Performance
pos	pos	30	RP
pos	neg	1	FN
neg	pos	2	FP
neg	neg	171	RN

Parameter	FT MTB
Sens (%)	96.8
Spec (%)	98.8

Table 3. Results FT MTB-PCR Non-respiratory specimens (n= 25)

Culture	Microscopy	FT MTB	n	Specimens
pos	pos	pos	1	Puncture Abscess
neg	neg	neg	4	Liquor, Puncture, Biopsy
neg	ND	neg	20	Urines

FluoroCycler

Table 4. Results CT MTB-PCR Respiratory specimens (n= 77)

n= 13 specimens: microscopic positive/scanty

Culture/Clinic	CT MTB	n	Performance
pos	pos	13	RP
pos	neg	0	FN

Parameter	CT MTB
Sens (%)	100

Table 5. Results CT MTB-PCR Respiratory specimens (n= 77)

n= 64 specimens: microscopic negative

Culture/Clinic	CT MTB	n	Performance
pos	pos	25	RP
pos	neg	1	FN
neg	pos	0	FP
neg	neg	38	RN

Parameter	CT MTB
Sens (%)	96.1
Spec (%)	100

CobasTaqMan

Conclusions

Both PCR- assays, the new **Fluoro**Type **MTB** and the Cobas TaqMan evaluated for the direct detection of *Mycobacterium tuberculosis* provide sensitive and specific results in about 3 hours.