

Evaluation of the **GenoType Mycobacterium CM 2.0** PCR-assay for the direct detection of mycobacterium species in patient specimens

Ulrich Eigner, Elvira Richter, Carolin Tauber, Rosemarie Schwarz, Martin Holfelder
Department of Infectious Diseases, Labor Limbach, Im Breitspiel 16, 69126 Heidelberg

Introduction and Purpose

For the direct detection of TB-bacteria in clinical specimens a wide series of assays are available and performance data is usually well documented. In contrast to this, no commercial assays are available for the rapid and direct detection of nontuberculous mycobacteria (NTM) in clinical specimens. We performed the first evaluation of the new commercially available **GenoType Mycobacterium CM 2.0** (Hain Lifescience, Nehren, Germany) molecular assay for the direct detection of 15 mycobacteria species, including the *M. tuberculosis*-complex directly in patient specimens.

Methods

A total of 76 specimens (n=70 respiratory, n=6 non-respiratory) with positive culture for mycobacteria or suspected mycobacterial infection were collected for the evaluation of the PCR-assay. Results of the **GenoType Mycobacterium CM 2.0** assay were compared to mycobacterial culture in liquid BACTEC MGIT medium (BD Biosciences, Heidelberg, Germany) and on solid culture media (Löwenstein-Jensen, Stonebrink; Thermo Fisher). Additionally, acid-fast bacilli (AFB) microscopy was taken into account. After decontamination of the specimens with the NALC-method, 500 µl of the final suspension was used for manual DNA isolation with the **GenoLyse**® kit (Hain Lifescience, Nehren, Germany).

The **GenoType Mycobacterium CM 2.0** assay is based on the line-probe technology. The assay was performed according to the manufacturer's instructions.

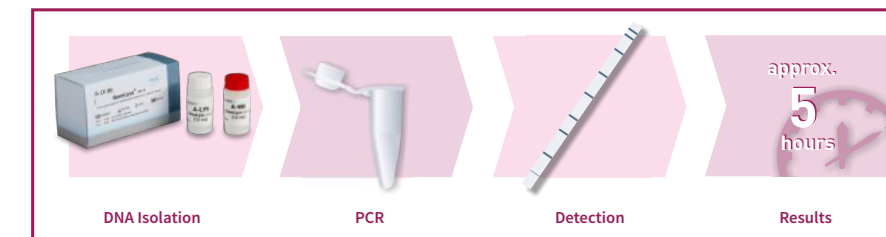


Fig. 1: Test procedure **GenoType Mycobacterium CM 2.0**

Conclusions

The **GenoType Mycobacterium CM 2.0** assay is the first commercial assay for the direct detection of the most relevant mycobacterial species directly in patient specimens. The **GenoType Mycobacterium CM 2.0** assay has proven to be a reliable method for rapid detection and identification of mycobacteria in smear-positive clinical specimens. Using PCR assays for screening of NTM in smear-negative specimens still is questionable due to lower sensitivity of assays and in particular due to the unclear clinical relevance of detection of DNA of ubiquitously present NTM.

GenoType Mycobacterium CM 2.0 PCR-positive / culture-positive				GenoType Mycobacterium CM 2.0 PCR-negative / culture-positive				GenoType Mycobacterium CM 2.0 PCR-positive / culture-negative			
Species	n	Smear result		Species	n	Smear result		Species	n	Smear result	
		positive	negative			positive	negative			positive	negative
<i>M. abscessus</i>	2	2	0	<i>M. avium</i>	2	0	2	<i>M. chelonae/M. intracellulare</i>	1	0	1
<i>M. avium</i>	4	4	0	<i>M. gordonae</i>	1	0	1	<i>M. gordonae</i>	2	0	2
<i>M. chelonae</i>	1	1	0	<i>M. intracellulare</i>	7	0	7	Mycobacterium species	1	0	1
<i>M. fortuitum</i>	1	1	0	<i>M. tuberculosis</i>	9	0	9	<i>M. tuberculosis</i>	3	1	2
<i>M. gordonae</i>	1	1	0	Total	19	0	19	Total	7	1	6
<i>M. intracellulare</i>	9	9	0								
<i>M. tuberculosis</i>	12	12	0								
<i>M. xenopi</i>	3	3	0								
Total	33	33	0								

Table 1: Results for the direct detection of mycobacteria species in patient specimens

Results

76 specimens were evaluated with the **GenoType Mycobacterium CM 2.0** PCR-assay for the direct detection of mycobacteria species in patient specimens. 33 samples were congruently positive for culture and PCR test. These included the following species: *M. abscessus* (n=2), *M. chelonae* (n=1), *M. fortuitum* (n=1), *M. avium* (n=4), *M. gordonae* (n=1), *M. intracellulare* (n=9), *M. tuberculosis* (n=12), *M. xenopi* (n=3). 22 of these specimens were AFB-positive. 19 specimens were culture-positive and PCR-negative (*M. avium* (n=2), *M. gordonae* (n=1), *M. intracellulare* (n=7), *M. tuberculosis* (n=9)). All of these specimens were smear-negative. 7 specimens had a positive PCR-result but culture remained negative (*M. tuberculosis* (n=3), known TB-patients), *M. gordonae* (n=2), *Mycobacterium species* (n=1), *M. chelonae / M. intracellulare* mixture (n=1). For 17 specimens culture and PCR remained negative (the results of the **GenoType Mycobacterium CM 2.0** assay are shown in Table 1).

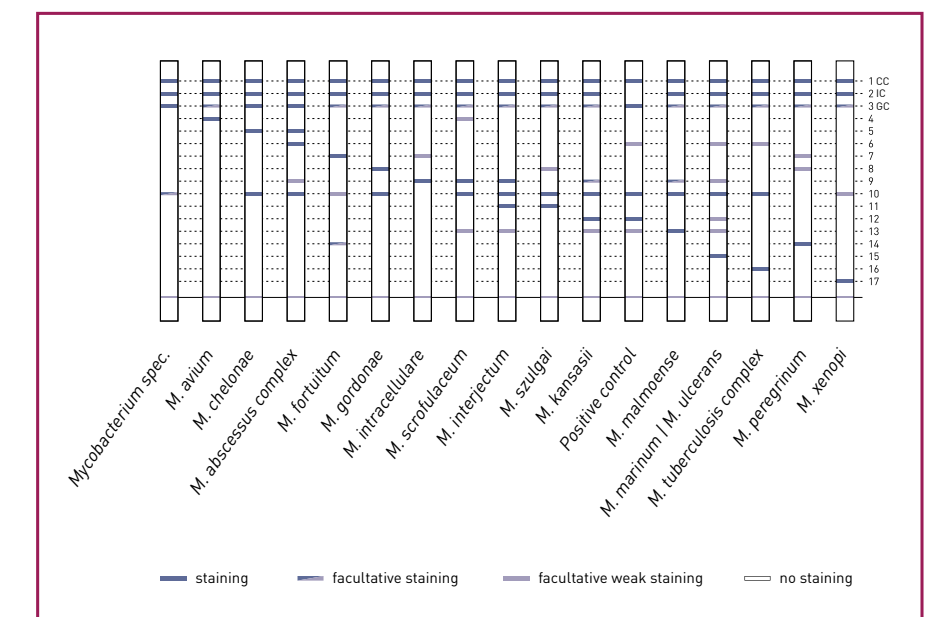


Fig. 2: Possible results with **GenoType Mycobacterium CM 2.0**