Identification of *Mycobacterium avium* and *Mycobacterium intracellulare/chimaera* in clinical practice using probe data of the Xpert MTB/RIF assay

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Introduction and purpose

Identification of non-tuberculosis mycobacteria (NTM) remains a major challenge in clinical practice, and requires specific tools such as sequencing, MALDI-TOF MS or commercial molecular assays for accurate diagnosis. *Mycobacterium avium* and *M. intracellulare* are regularly involved in human infections, particularly among severely immunocompromised patients. *M. chimaera* has recently been reported as a cause of health-care associated endocarditis, and is very similar genetically to *M. intracellulare*. In our clinical practice, we performed Xpert MTB/RIF© PCR on all MGIT cultures after confirmation of the presence of a mycobacterium by fluorescence microscopy. The principle of this test is based on the amplification of the Rifampicin Resistance Determining Region (RRDR) and the binding of 5 different segments by molecular-beacon probes names A, B, C, D and E. In our experience, approximately 50% of positive cultures are negative for *M. tuberculosis* (MTB), and identification of NTM is performed by *rpoB* sequencing or MALDI-TOF MS.

Methods: We analysed the raw Probe results of 31 Xpert MTB/RIF tests performed on positive MGIT cultures for which an NTM was later confirmed and at least one Probe was positive (A, B, C, D or E).

Results: All results could be classified within 3 different profiles determined by which of the 5 probes were positive. Among the 8 strains presenting an amplification of Probe A only, all were identified as *M. intracellulare, M. chimaera* or *M. marseillense*, three closely related species. Among the 14 strains which presented an amplification of Probe C only, different species were identified including *M. gordonae* (8), *M. xenopi* (3), *M. simiae* (1), *M. fortuitum* (1) and *M. avium* (1). Among the 9 strains presenting an amplification of both Probe C and Probe E, all were identified as *M. avium*.

Conclusions: In our clinical experience, the analysis of Xpert MTB/RIF probe results allowed a correct identification of 3 important pathogenic NTM. These results were supported by the fact that there is a partial overlap of the RRDR region of the *rpoB* gene of MTB with other mycobacterial species. Further studies would be needed to better understand the clinical value of this observation.

Isolate #	Probe profile	Probe A	ctA	EndPointA	Probe B	Probe C	ctC	EndPointC	Probe D	Probe E	ctE	EndpointE	RPOB-ID	MALDI-TOF MS	SCORE
1	A	Pos	33,2	144	Neg	Neg			Neg	Neg			-	*M. chimaera_intracellulare_group*	2142
2	A	Pos	33,5	116	Neg	Neg			Neg	Neg			*M. chimaera*	*M. chimaera_intracellulare_group*	2335
3	A	Pos	27,2	100	Neg	Neg			Neg	Neg			*M. chimaera*	*M. chimaera_intracellulare_group*	2314
4	A	Pos	29,3	122	Neg	Pos			Neg	Neg			*M. marseillense*	*M. marseillense*	2138
5	A	Pos	35,2	82	Neg	Neg			Neg	Neg			*M. intracellulare*	*M. chimaera_intracellulare_group*	2257
6	A	Pos	27,5	102	Neg	Neg			Neg	Neg			*M. chimaera*	-	-
7	A	Pos	27,9	103	Neg	Neg			Neg	Neg			*M. chimaera_intracellulare_group*	*M. chimaera_intracellulare_group*	2042
8	A	Pos	30,9	93	Neg	Neg			Neg	Neg			*M. chimaera_intracellulare_group*	*M. chimaera_intracellulare_group*	2042
9	C	Neg			Neg	Pos	27,7	43	Neg	Neg	0	-4	*M. xenopi*	*M. xenopi*	1566
10	C	Neg			Neg	Pos	29,6	160	Neg	Neg	0	1	-	*M. gordonae*	2056
11	C	Neg			Neg	Pos	29,7	76	Neg	Neg	0	-1	-	*M. gordonae*	1732
12	C	Neg			Neg	Pos	28,2	37	Neg	Neg	0	-8	*M. xenopi*	*M. xenopi*	1716
13	C	Neg			Neg	Pos	29,2	96	Neg	Neg	0	10	-	*M. gordonae*	1941
14	C	Neg			Neg	Pos	39,6	26	Neg	Neg	0	-1	*M. simiae*	*M. simiae*	-
15	C	Neg			Neg	Pos	29,9	34	Neg	Neg	0	-1	-	*M. fortuitum*	2313
16	C	Neg			Neg	Pos	29	133	Neg	Neg	0	-4	-	*M. gordonae*	1930
17	C	Neg			Neg	Pos	29	122	Neg	Neg	0	-2	*M. gordonae*	*M. gordonae*	1300
18	C	Neg			Neg	Pos	29,2	109	Neg	Neg	0	-3	*M. gordonae*	-	-
19	C	Neg			Neg	Pos	28,6	28	Neg	Neg	0	-7	*M. xenopi*	-	-
20	C	Neg			Neg	Pos	25,1	101	Neg	Neg	0	1	*M. gordonae*	*M. gordonae*	1777
21	C	Neg			Neg	Pos	27	86	Neg	Neg	0	6	*M. gordonae*	*M. gordonae*	1500
22	C	Neg			Neg	Pos	35	33	Neg	Neg	0	7	*M. avium*	*M. avium*	1939
23	CE	Neg			Neg	Pos	31,9	58	Neg	Neg -> Pos	0	21	*M. avium*	*M. avium*	2270
24	CE	Neg			Neg	Pos	31,5	51	Neg	Neg -> Pos	0	23	*M. avium*	*M. avium*	1941
25	CE	Neg			Neg	Pos	26,9	50	Neg	Pos	35,8	28	*M. avium*	*M. avium*	2017
26	CE	Neg			Neg	Pos	34,9	67	Neg	Neg -> Pos	0	22	*M. avium*	*M. avium*	1842
27	CE	Neg			Neg	Pos	34,1	60	Neg	Neg -> Pos	0	23	-	*M. avium*	2061
28	CE	Neg			Neg	Pos	34,6	54	Neg	Neg -> Pos	0	23	-	*M. avium*	1840
29	CE	Neg			Neg	Pos	30,9	52	Neg	Pos	38,5	28	-	*M. avium*	1717
30	CE	Neg			Neg	Pos	33,9	52	Neg	Neg -> Pos	0	17	*M. avium*	-	-
31	CE	Neg			Neg	Pos	34	43	Neg	Neg -> Pos	0	15	*M. avium*	*M. avium*	-

Table 1: Raw probe results obtained using the Xpert MTB/RIF assay on clinical non-tuberculosis mycobacterial species.