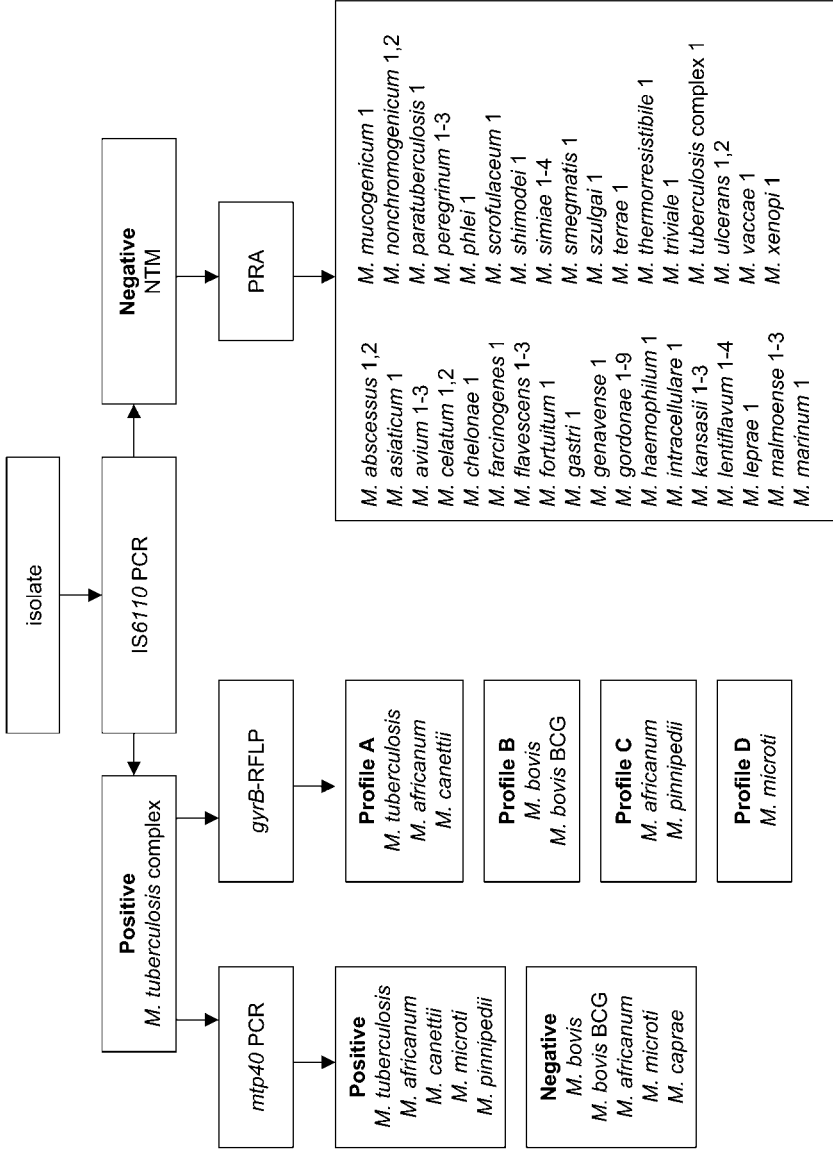


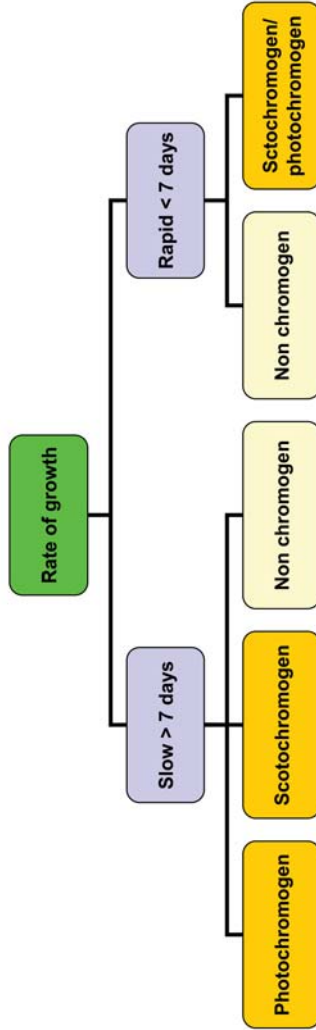
ANNEX 1

FLOWCHARTS

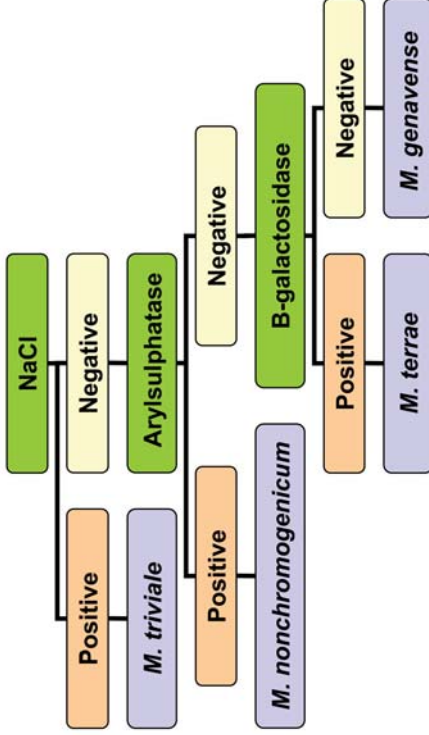
MOLECULAR ID FLOWCHART



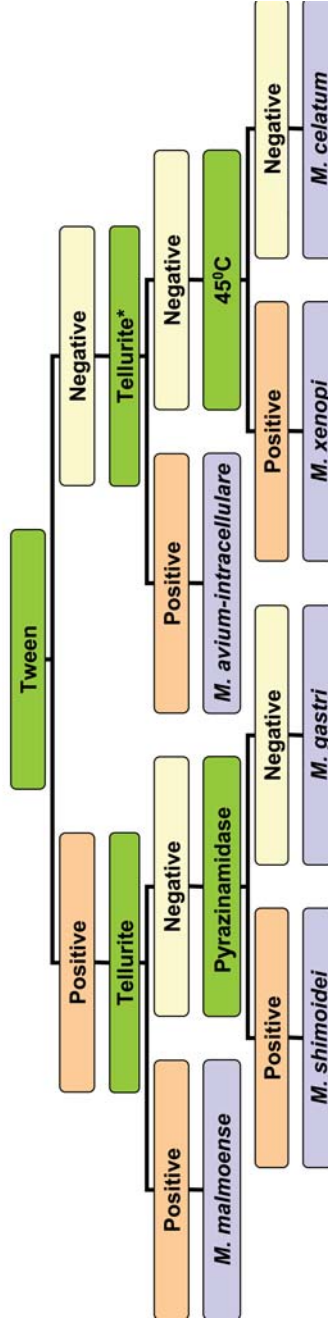
Initial Classification of NTM According to Rate of growth and Pigment Production



Non-chromogen Slow Growers Catalase and Tween Hydrolysis Positive

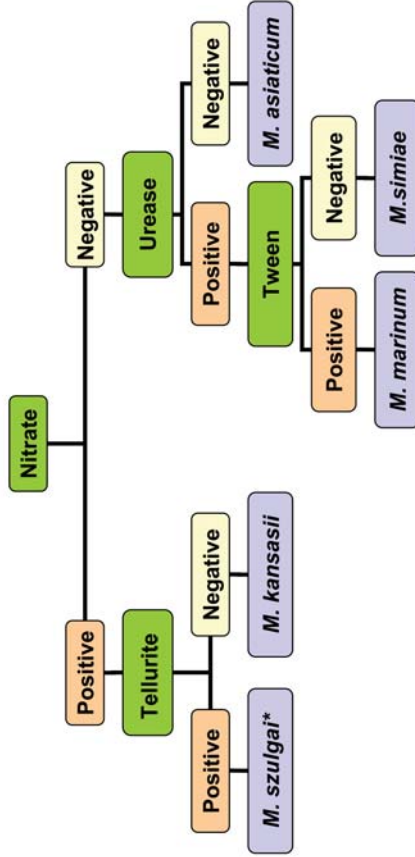


Non-chromogen Slow Growers Catalase and Nitrate Reduction Negative



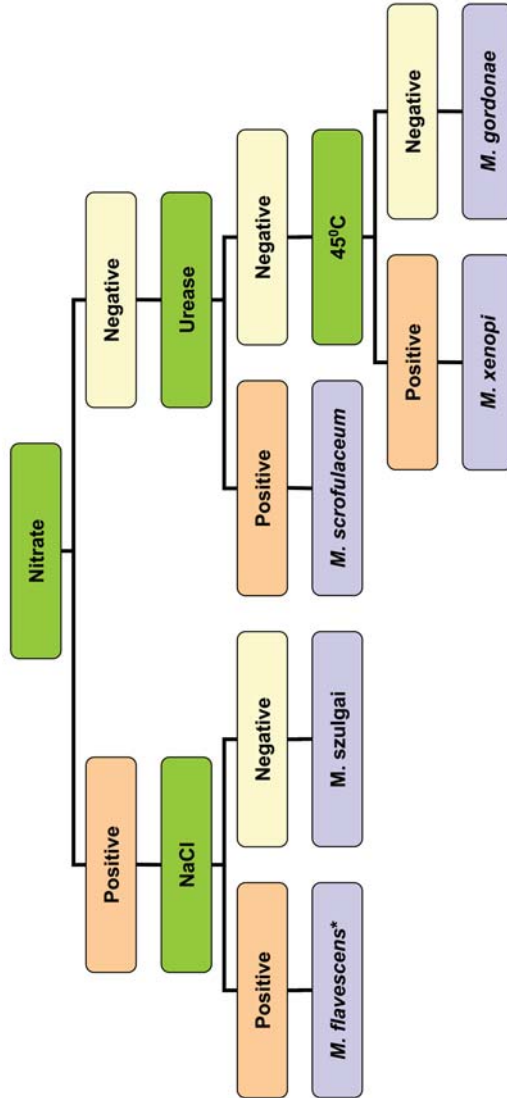
Note* *M. xenopi*, can be scotochromogen, tellurite negative at 3 days and positive at 9 days

Photochromogen Slow Growers



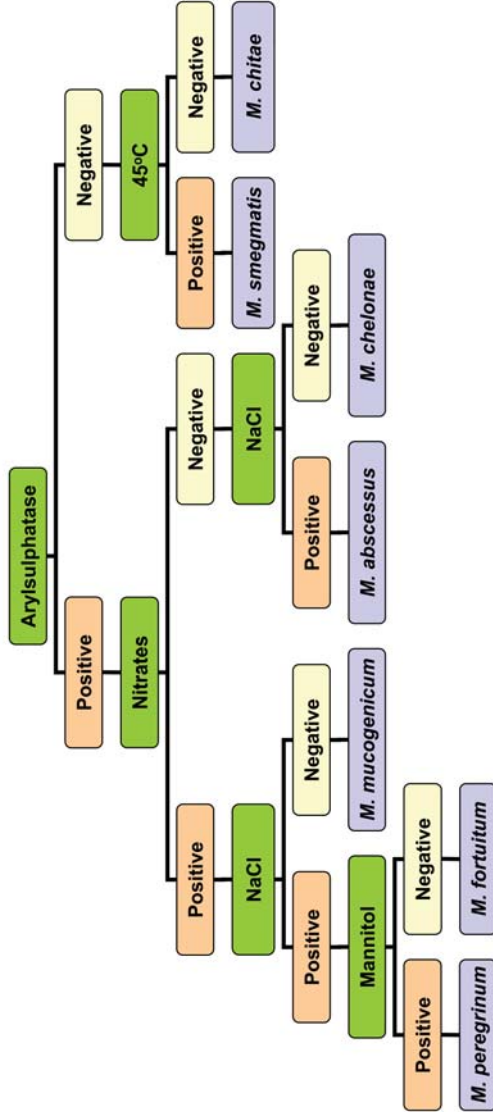
Note*: *M. szulgai*, is photochromogen at 25°C

Scotochromogen Slow Growers



* *M. flavescens* can be identified as a rapid grower

Non-chromogen Rapid Growers



ANNEX 2

TABLES

MOST USEFUL TESTS FOR IDENTIFICATION OF FREQUENTLY ISOLATED NTM

SPECIES	25°C	37°C	45°C	NaCl	HA	INH	Cat SQ	Nitrate	Urease	PZA	Arylsulfatase		Phos	B-gal	Tween	Tellurite	
											3d	14d				3d	9d
SLOW GROWER NONCHROMOGENS																	
<i>M. avium</i>	+/-	+	-	-	+	+	-	-	-	+	-	-	-	-	-	+	+
<i>M. gastri</i>	+	+	-	-	+/-	-	-	-	+/-	-	-	-	+	-	+	-	-
<i>M. intracellulare</i>	+	+	+/-	-	+	+/-	-	-	-	+	-	+/-	-	-	+	+	+
<i>M. malmoense</i>	+	+	-	-	+	+	-	-	+/-	+	-	-	-	-	+	+	+
<i>M. nonchromogenicum</i>	+	+	-	-	+	+	+	-	-	+/-	-	+	+	+	+	-	-
<i>M. shimoides</i>	-	+	-/+	-	-	-	-	-	-	-	-	-	+	-	+	-	-
<i>M. terrae</i>	+	+	-	-	+	+	+	+/-	-	+/-	-	-	+	+	+	-	-
<i>M. triviale</i>	+	+	-	+	ND	+	+	-	-	+/-	-	+/-	+	-	+	-	+
<i>M. celatum</i> **	-	+	-	-	ND	ND	-	-	-	+	-	+	+	ND	-	-	-
SLOW GROWER PHOTOCROMOGENS																	
<i>M. xenopi</i> ***	-	+	+	+	+	+/-	-	-	-	+/-	+	+	-	-	-	-	+
<i>M. kansasii</i>	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-
<i>M. marinum</i>	+	+	-	-	ND	+/-	-/+	-	+	+	+	+	+	-	+	-	-
<i>M. simiae</i>	+	+	-	-	+	+/-	+	-	+	+	-	-	-	-	-	+	+
SLOW GROWER SCOTOCROMOGENS																	
<i>M. flavescens</i>	+	+	+/-	+	ND	-	+	+	+	+	-	+	-	-	+	+	+
<i>M. goodae</i>	+	+	-	-	ND	-	-	-	-	+/-	-	-	+	-	+	-	-
<i>M. scrofulaceum</i>	+	+	-	-	+	+	+	-/+	+	+	-	-	-	-	-	-	-
<i>M. szulgai</i> ****	+	+	-	-	ND	+	+	+	+	+	-	+/-	+	-	+/-	+	+/-

* Requires hemin for growth

** pale yellow

*** N/P

**** P at 25°C

Symbols

+	> 85% of strains positive	R	rapid	HA	hydroxylamine
-	<15% of strains negative	M	moderate	INH	isoniazid
+/-	50 to 85% of strains positive	S	slow	Cat SQ	catalase semiquantitative
-/+	15 to 49% of strains positive	N	nonchromogenic	PZA	pyrazinamide
		Sc	scotochromogenic	Phos	Phosphatase
		P	photochromogenic	B-gal	beta-galactosidase
		ND	no data		

MOST USEFUL TESTS FOR IDENTIFICATION OF FREQUENTLY ISOLATED NTM

RAPID GROWERS

species	Pigm	25°C	37°C	45°C	NaCl	Picric	Phos	nitrate	Aryl 3d	HA	B-gal	Iron	Tween	inositol	mantol	citrate
<i>M. fortuitum</i>	N	+	+	-	+	+	+	+	+	+	-	+	+	-	-	-
<i>M. peregrinum</i>	N	+	+	-	-/+	+	+	+	+	+	-	+	+	-	+	-
<i>M. chelonae</i>	N	+	+/-	-	-	-/+	+	-	+	+	+	-	-/+	-	-	+
<i>M. abscessus</i>	N	+	+	-	+	+	+	-	+	+	-	-	-/+	-	-	-
<i>M. mucogenicum</i>	N	ND	+	ND	-	ND		+/-	+		-	ND	+			
<i>M. smegmatis</i>	N	+	+	+	+	+	-	+	-	-	-	+	+	+	+	+
<i>M. phlei</i>	S	+	+	+	+	+	+	+	-	-	-	+	+	-	+	+
<i>M. vaccae</i>	S/P	+	+	-	+	+	-	+	-/+	-	-	+	+	+	+	+
<i>M. parafortuitum</i>	S/P	+	+	-	+/-	+	-	+/-	+/-	-	-	+	ND		+	+

Symbols

- + > 85% of strains positive
- <15% of strains negative
- +/- 50 to 85% of strains positive
- /+ 15 to 49% of strains positive

- R rapid
- M moderate
- S slow
- N non chromogenic
- Sc scotochromogenic
- P Photochromogenic
- ND no data

- HA hidroxilamine
- Phos Phosphatase
- B-gal beta-galactosidase
- Aryl 3d arylsulphatase 3d

slow grower species	Pigment	25°C	37°C	45°C	TCH	NaCl	PNB	Picric	HA
<i>M. tuberculosis</i>	N	-	+	-	+	-	-	-	-
<i>M. bovis</i>	N	-	+	-	-	-	-	-	-
<i>M. africanum</i>	N	-	+	-	-	-	-	-	-
<i>M. microti</i>	N	-	+	-	-	-	-	-	-
<i>M. avium</i>	N	+/-	+	-	+	-	+	-	+
<i>M. gastri</i>	N	+	+	-	+	-	+/-	-	+/-
<i>M. genavense</i>	N	+/-	+	-	+	-	ND	-	ND
<i>M. haemophilum**</i>	N	+	+	-	+	-	-	-	+
<i>M. intracellulare</i>	N	+	+	+/-	+	-	+	-	+
<i>M. malmoense</i>	N	+	+	-	+	-	+	-	+
<i>M. nonchromogenicum</i>	N	+	+	-	+	-	+	-	+
<i>M. paratuberculosis</i>	N	-	+	-	+	-	ND	ND	ND
<i>M. shimoidei</i>	N	-	+	-/+	+	-	+	-	-
<i>M. terrae</i>	N	+	+	-	+	-	+	-	+
<i>M. triviale</i>	N	+	+	-	+	+	+	-	ND
<i>M. ulcerans</i>	N	+/-	-	-	+	-	ND	-	+
<i>M. celatum</i>	N*	-	+	-	+	-	ND	ND	ND
<i>M. xenopi</i>	N/P	-	+	+	+	-	+/-	-	+
<i>M. asiaticum</i>	P	+	+	-	+	-	+	-	+
<i>M. intermedium</i>	P	+	+	-	ND	ND	ND	ND	ND
<i>M. kansasii</i>	P	+	+	-	+	-	+/-	-	-
<i>M. marinum</i>	P	+	+	-	+/-	-	+	-	ND
<i>M. simiae</i>	P	+	+	-	+	-	+	-	+
<i>M. cookii</i>	S	+/-	+	-	+	-	+	-	ND
<i>M. flavescens</i>	S	+	+	+	+	+	+	+	ND
<i>M. farcinogenes</i>	S			-	+	-	ND	-	ND
<i>M. gordonae</i>	S	+	+	-	+	-	+	-	ND
<i>M. interjectum</i>	S	+	+	-	+	-	ND	ND	ND
<i>M. scrofulaceum</i>	S	+	+	-	+	-	+	-	+
<i>M. szulgai</i>	S/P	+	+	-	+	-	+	-	ND

* pale yellow

**Requieres hemin for growth

Symbols

- +: > 85% of strains positive
- : <15% of strains negative
- +/-: 50 to 85% of strains positive
- /+ : 15 to 49% of strains positive
- R: rapid
- M: moderate
- S: slow
- N: non chromogenic
- Sc: scotochromogenic
- P: photochromogenic
- ND: no data

	INH	Niacin	Catalase 68°C	Catalase SQ	Nitrate	Urease	PZA	Aryl 3d	Aryl 14d	phosphatase	B-gal	Tween	Tellurite 3d	Tellurite 9d
	-	+	-	-	+	+	+	-	-	-	-	+/-	-	+
	-	-	-	-	-	+	-	-	-	-	-	+/-	-	+
	-	+/-	-	-	+/-	+	+/-	-	-	+/-	-	+/-	-	-
	-	+	-	-	+/-	+	+	-	-	-	-	+/-	-	-
	+	-	+/-	-	-	-	+	-	-	-	-	-	+	+
	-	-	-	-	-	+/-	-	+/-	-	+	-	+	-	-
	ND	-	+	+	-	+/-	+	-	-	-	ND	+/-	ND	ND
	+	-	-	-	-	-	+	-	-	-	-	-	-	-
	+/-	-	+	-	-	-	+	+/-	+/-	-	-	-	+	+
	+	-	+	-	-	+/-	+/-	-	-	-	-	+	+	+
	+	-	+	+	-	-	+/-	+	+	+	+	+	-	-
	ND	-	+/-	-	-	-	+	-	-	-	-	+/-	ND	ND
	-	-	+/-	-	-	-	+	-	-	+	-	+	-	-
	+	-	+	+	+/-	-	+/-	-	-	+	+	+	-	-
	+	-	+	+	+	-	+/-	+/-	+/-	+	-	+	-	+
	+	-	+	-	-	+/-	-	-	+	+/-	-	-	-	-
	ND	-	+	-	-	-	+	+	+	+	ND	-	-	-
	+/-	-	+/-	-	-	-	+/-	+	+	-	-	-	-	+
	+/-	-	+	+	-	-	-	-	-	+	-	+	-	-
	ND	-	+	ND	+	+	-	ND	ND	+	ND	+	ND	ND
	-	-	+	+	+	+	+	+	+	+	-	+	-	-
	+/-	-	+/-	-/+	-	+	+	+	+	+	-	+	-	-
	+/-	+	+	+	-	+	+	-	-	-	-	-	+	+
	-	-	-	+	+	-	-	+	+	+	ND	-	-	-
	-	-	+	+	+	+	+	+	+	-	-	+	+	+
	-	-	-	+	+	+/-	ND	ND	ND	-	ND	+	ND	ND
	-	-	+	+	-	-	+/-	-	-	+/-	-	+	-	-
	ND	-	+	-/+	-	+	+	ND	ND	-	ND	-	-	-
	+	-	+	+	-/+	+	+	-	-	-	-	-	-	+
	+	-	+	+	+	+	+	+/-	+/-	+	-	+/-	+/-	+/-

Conventions

Pigm: pigmentation
 25°C: growth at 25°C
 37°C: growth at 37°C
 45°C: growth at 45°C
 TCH: 2-thiophene carboxylic acid hydrazide 5 mg/mL
 NaCl: growth in NaCl 5%
 PNB: growth in p-nitro benzoic acid 0.5 mg/mL
 Picric: growth in picric acid 0,2%
 INH: growth in the presence of isoniazid 10 µg/mL
 Acid phosphatase:
 Catalase at 68°C
 Catalase SQ: catalase semiquantitative
 Nitrate: reduction of nitrates to nitrites

PZA: pyrazinamidase
 HA: hydroxylamine
 B-gal: beta galactosidase
 Iron: iron uptake
 Tween: tween 80 hydrolysis
 Tellurite 3d: reduction of tellurite at 3 days
 Tellurite 9d: reduction of tellurite at 9 days
 Inositol: utilization of inositol
 Manitol: utilization of manitol
 Citrate: utilization of citrate
 Aryl 3d: Arylsulfatase at 3 days
 Aryl 14d: Arylsulfatase at 14 days
 Phosphatase: acid phosphatase

rapid grower species	Pigm	25°C	37°C	45°C	TCH	NaCl	PNB	Picric	INH	
<i>M. fortuitum</i>	N	+	+	-	+	+	+	+	+	
<i>M. peregrinum</i>	N	+	+	-	+	-/+	+	+	+	
<i>M. chitae</i>	N	+	+	-	+	+	+	+	ND	
<i>M. chelonae</i>	N	+	+/-	-	+	-	+	-/+	ND	
<i>M. abscessus</i>	N	+	+	-	+	+	+	+	ND	
<i>M. mucogenicum</i>	N	ND	+	ND	+	-	+	ND	ND	
<i>M. smegmatis</i>	N	+	+	+	+	+	+	+	ND	
<i>M. phlei</i>	S	+	+	+	+	+	+	+	ND	
<i>M. vaccae</i>	S/P	+	+	-	+	+	+	+	ND	
<i>M. parafortuitum</i>	S/P	+	+	-	+	+/-	+	+	ND	
<i>M. thermoresistibile</i>	S	+	+	+		+		+		
<i>M. duvalli</i>	S	+	+	-	+	ND	+	ND	ND	

Symbols

- +: > 85% of strains positive
- : <15% of strains negative
- +/-: 50 to 85% of strains positive
- /+ : 15 to 49% of strains positive
- R: rapid
- M: moderate
- S: slow
- N: non chromogenic
- Sc: scotochromogenic
- P: photochromogenic
- ND: no data

	phosphatase	Catalase 68°C	Catalase SQ	nitrate	Urease	PZA	Aryl 3d	HA	B-gal	Iron	Tween	Tellurite 3d	inositol	manitol	citrate
	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-
	+	+	+	+	+	+	+	+	-	+	+	+	-	+	-
	+	+	+	+	+	+	-	-	-	-	+	+	ND	-	-
	+	+/-	+	-	+	+	+	+	+	-	-/+	+	-	-	+
	+	-+	+	-	+	+	+	+	-	-	-/+	+	-	-	-
		ND	+	+/-	+	ND	+		-	ND	+	ND			
	-	-	-	+	+	ND	-	-	-	+	+	ND	+	+	+
	+	+	+	+	+	ND	-	-	-	+	+	ND	-	+	+
	-	+	+	+	+	ND	-/+	-	-	+	+	ND	+	+	+
	-	+	+	+/-	ND	ND	+/-	-	-	+	ND	ND	ND	+	+
	-			+			-	-		-	+		-	-	-
	-	ND	ND	+	ND	ND	-	-	-	ND	ND	ND	ND	+	-

Conventions

Pigm: pigmentation

25°C: growth at 25°C

37°C: growth at 37°C

45°C: growth at 45°C

TCH: 2-thiophene carboxylic acid hydrazide 5 mg/mL

NaCl: growth in NaCl 5%

PNB: growth in p-nitro benzoic acid 0.5 mg/mL

Picric: growth in picric acid 0.2%

INH: growth in the presence of isoniazide 10 m/mL

Acid phosphatase:

Catalase at 68°C

Catalase SQ: catalase semiquantitative

Nitrate: reduction of nitrates to nitrites

PZA: pirazinamidase

HA: hydroxylamine

B-gal: beta galactosidase

Iron: iron uptake

Tween: tween 80 hydrolysis

Tellurite 3d: reduction of tellurite at 3 days

Inositol: utilization of inositol

Manitol: utilization of manitol

Citrate: utilization of citrate

Aryl 3d: Arylsulfatase at 3 days

ANNEX 3

CD - INTERACTIVE TABLES IN EXCEL FORMAT Instructions for using Excel table with filters for phenotypic identification

The Excel file called “phenotypic tests for identification of mycobacterial species” has two sheets, slow growers and rapid growers. To start identification using the tables it is necessary to have previously the results for rate of growth and pigmentation, followed by results of the other phenotypic tests.

1. The table is arranged as a list of species in column A and a list of phenotypic tests as headings in the other columns (B, C, D...etc.); consequently each species has a result for each phenotypic test displayed.
2. Each column in the table has a heading with the name of the phenotypic test, and a small arrowhead; clicking with the pointer on the arrowhead gives you a list of the different options for that column
3. Select an option with the pointer (according to the results you have obtained for that specific test) and the table will display only the species with that specific result
4. Repeat the procedure for each phenotypic test for the species that you are identifying
5. You should end up with only one species or a small group of species, according to the results of your phenotypic tests

Notes:

- The number of phenotypic tests performed are in direct relation to the capability of the table for discriminating between species
- The results displayed in the table for each phenotypic test are a summary of the information published in the scientific literature. Some results may show discrepancies between different sources. When this was the case a consensus was obtained, prevailing the data from sources showing a larger number of strains tested.

Instructions for using Excel table with filters for molecular identification (PRA)

1. The table is arranged as a list of species in column A. Columns B, C and D display the first three possibilities of bands obtained after digestion with *BstE* II, and columns E, F, G and H display the possibilities of bands after digestion with *Hae* III.
2. The heading of each column shows a small arrowhead, clicking with the pointer on the arrowhead you have a list of the different options
3. Select an option according to your results, beginning with the *BstE* II bands and then the *Hae* III bands
4. After selecting all your results, you should end up with only one species.