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toute une vie en bonne santé

NTM: diagnostic & epidemiology

Samira Boarbi

National Reference Laboratory
Tuberculosis & Mycobacteria

SBIMC-BVIKM SYMPOSIUM 24th May 2022

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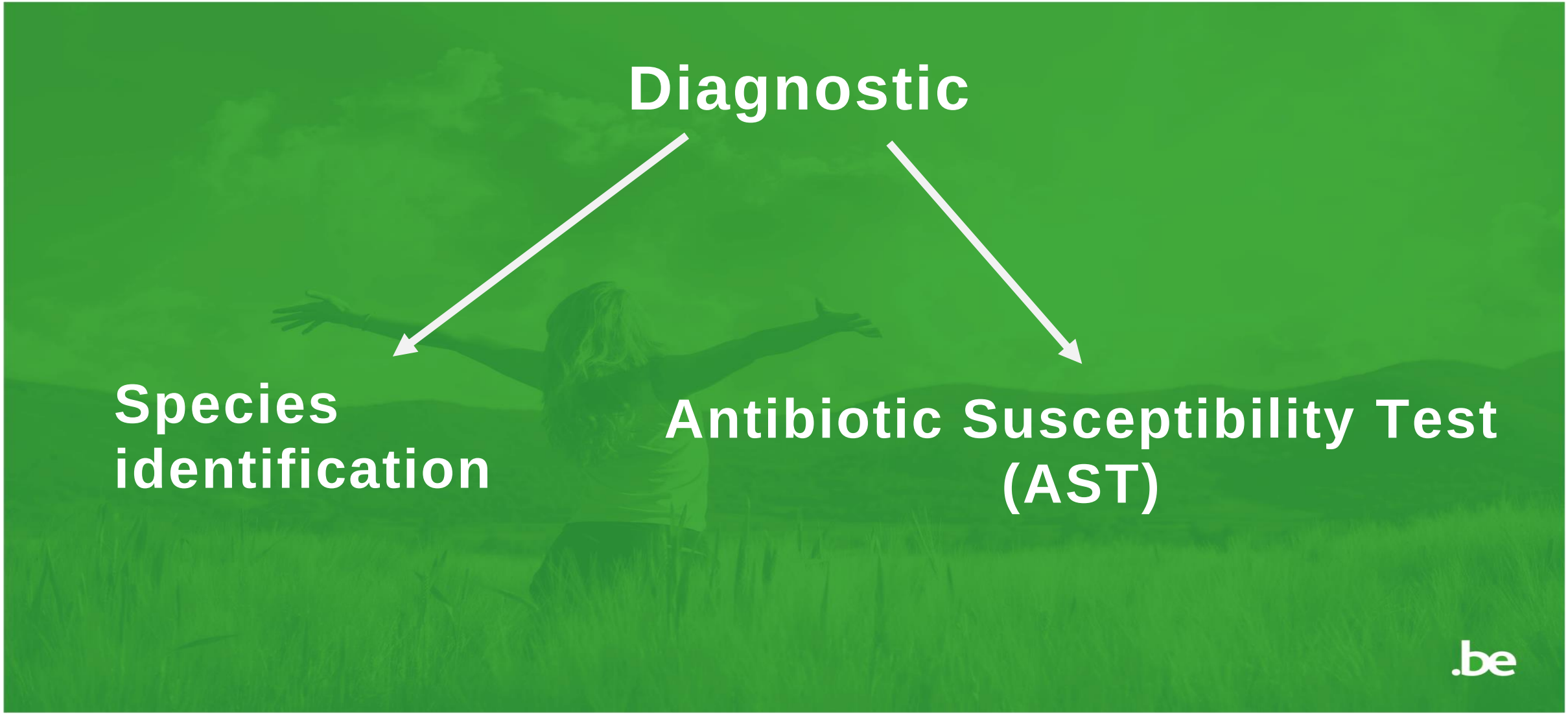


> 200 mycobacterial species

Strictly pathogenic	Potentially pathogenic or opportunist	Non (or rarely) pathogenic	
Slowly growing	**Slowly growing**	**Slowly growing**	
M. tuberculoiss	*M.asiaticum*	*M.cookii*	
M.bovis	*M.avium*	*M.gastri*	
M.africanum	*M.celatum*	*M.gordonae*	
M.ulcerans	*M.farcinogenes*	*M.hiberniae*	
M. microti	*M.genavense*	*M.nonchromogenicum*	
M. leprae	*M.haemophilum*	*M.terrae*	
***M. tuberculosis* complex**	*M.interjectum*	*M.triviale*	
	M.intermedium		
	M.intracellulare	**Rapidly growing**	
	M.kansasii	*M.agri*	*M.morokaense*
	M.lentiflavum	*M.aichiense*	*M.neoaurum*
	M.lepraemurium	*M.alvei*	*M.obuense*
	M.malmoense	*M.aurum*	*M.parafortuitum*
	M.marinum	*M.austroafricanum*	*M.phlei*
	M.paratuberculosis	*M.brumae*	*M.poriferae*
	M.scrofulaceum	*M.chitae*	*M.pulveris*
	M.shimoidei	*M.chubuense*	*M.rhodesiae*
	M.simiae	*M.confluentis*	*M.shangaiense*
	M.szulgai	*M.diernhoferi*	*M.smegmatis*
	M.xenopi	*M.duvalii*	*M.sphagni*
		M.fallas	*M.thermoresistibile*
	Rapidly growing	*M.flavescens*	*M.tokaiense*
	M.abscessus	*M.gadium*	*M.vaccae*
	M.chelonae	*M.gilvum*	*M.yunnanense*
	M.fortuitum	*M.komossense*	
	M.peregrinum	*M.madagascariense*	
	M.porcinum	*M.methylovorum*	

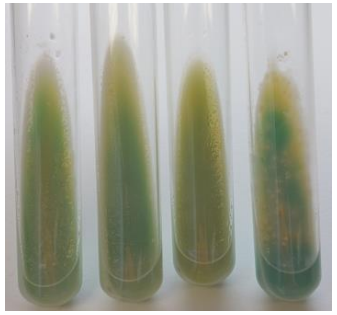
Non tuberculosis mycobacteria (NTM)

- **Slowly growing**
- **Rapidly growing**

Diagnostic

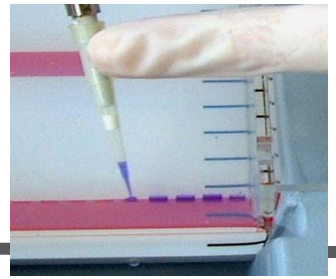
DNA preparation (Thermolysate)

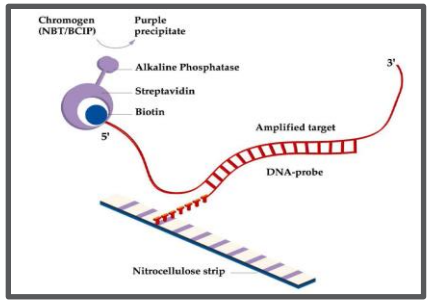
Sub-culture on LJ tubes

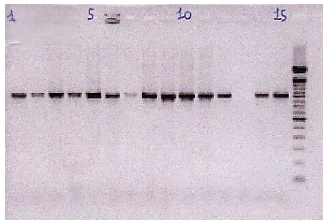


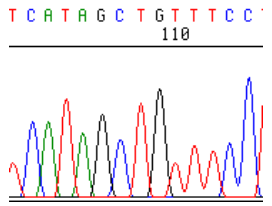
Molecular tests

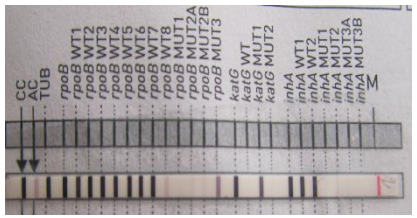
- ✓ PCR
- ✓ PCR + sequencing
- ✓ PCR + hybridization (LPA)











Species identification

Detection of mutations associated with the AB resistance

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Cultures on solid medium

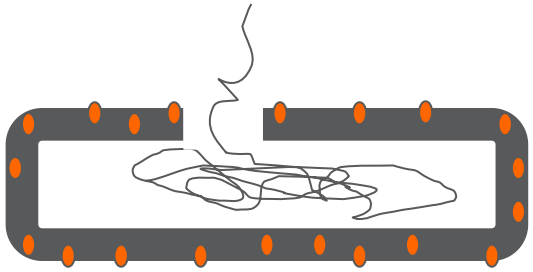
Different media:

Löwenstein-Jensen (LJ)
Coletsos
Middlebrook 7H10 and 7H11

Some mycobacterial species can be distinguished according to the aspect/pigmentation of the culture

Positif : 4-6 weeks
Macroscopic examination possible

Thermolysate : DNA preparation



Culture aliquote at 95° C 30 min

Thermolysate (= inactivated culture, DNA preparation)

PCR + sequencing

PCR amplification of region of interest

Purification Preparation

Sequencing

16SrRNA : species identification

CGG ATA GG-ACCA CGG GAT TCA TG TCC- TGT	Séquence déterminée
CGG ATA GG-ACCA CGG GAT GCA TG TCT- TGT	*M. tuberculosis*
CGG ATA GG-ACC**T** C**AA** GA**C** GCA TG TCT- T**C**T	*M. avium*
CGG ATA GG-ACCA CGG GAT **T**CA TG TC**C**- TGT	***M. marinum***
CGG ATA GG-ACC**T** **TTA** G**GC** GCA TG TCT- T**TA**	*M. intracellulare*
CGG ATA GG-ACCA C**TT** G**GC** GCA TG **C**CT- TGT	*M. gastri*
CGG ATA GG-ACCA C**TT** G**GC** GCA TG **C**CT- TGT	*M. scrofulaceum*
CGG ATA GG-ACCA C**TT** G**GC** GCA TG **C**CT- TGT	*M. simiae*
CGG ATA GG-ACC**T** C**TC** G**GC** GCA TG **C**CT**A** G**GA**	*M. intermedium*

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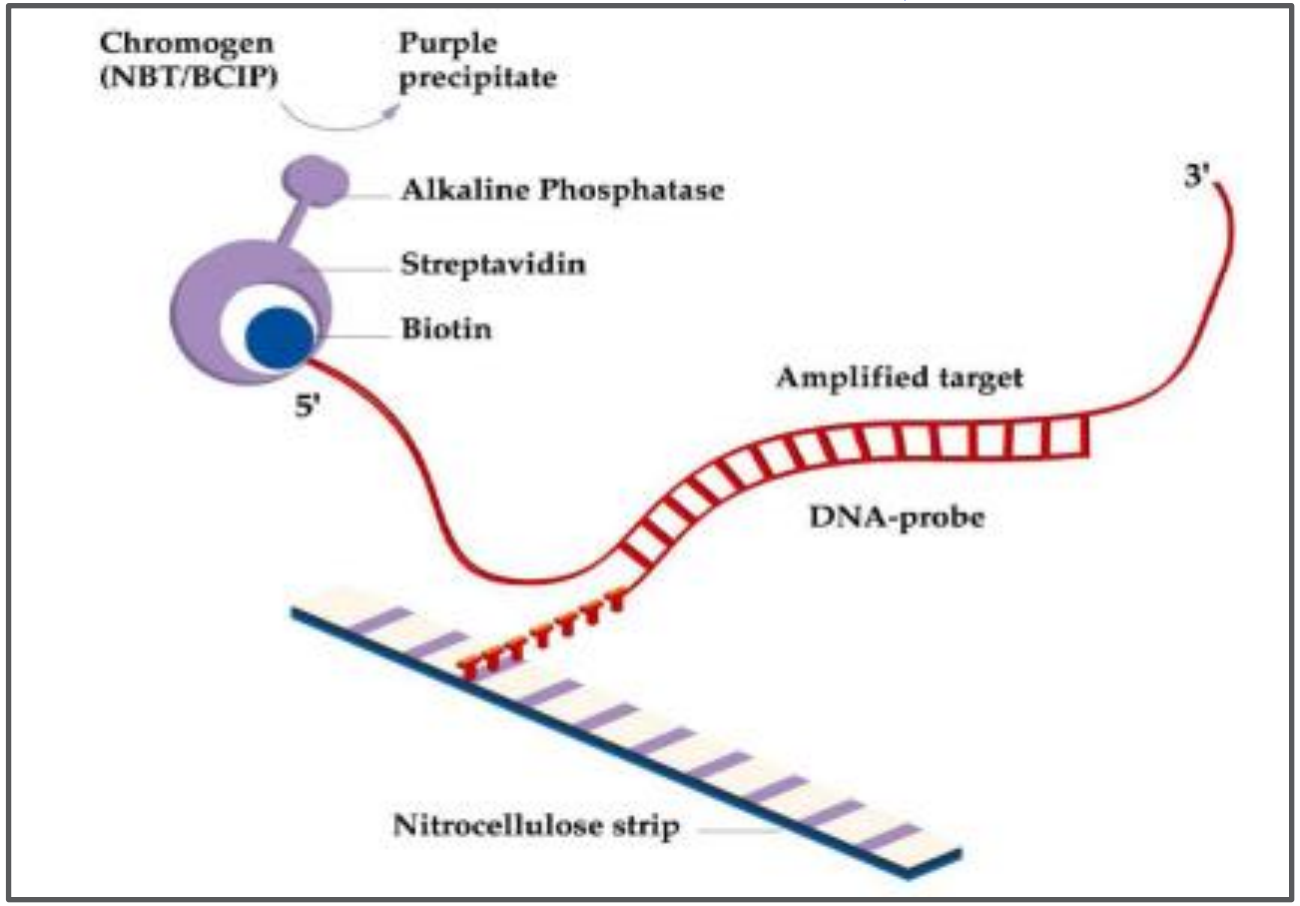
PCR + hybridization = LPA (Line Probe assay)

PCR amplification of region of interest → Separation of the 2 DNA strands by heating → Hybridization on membrane coated with probes

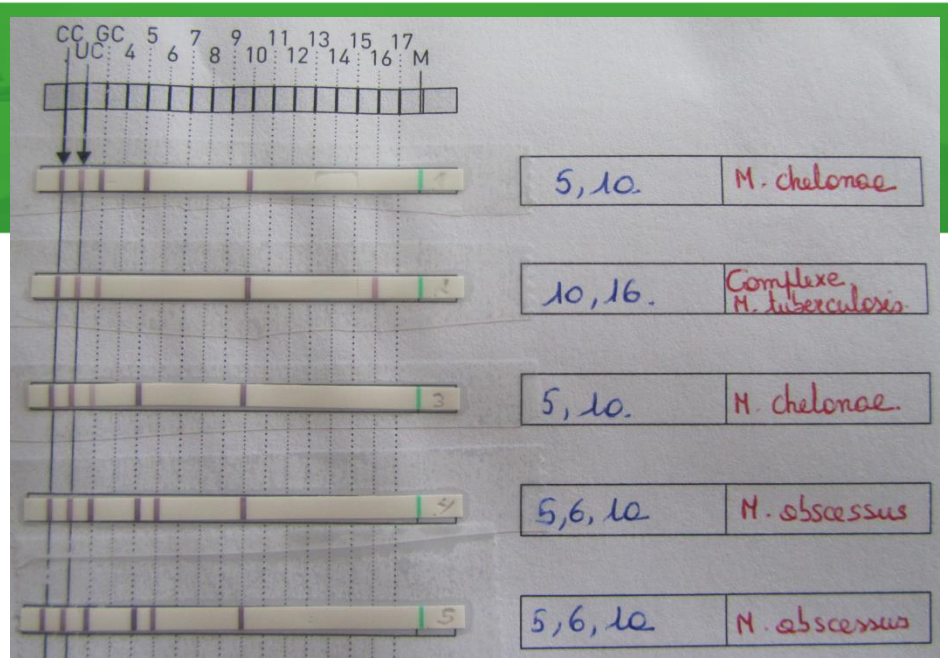


Commercial kits

Species Identification

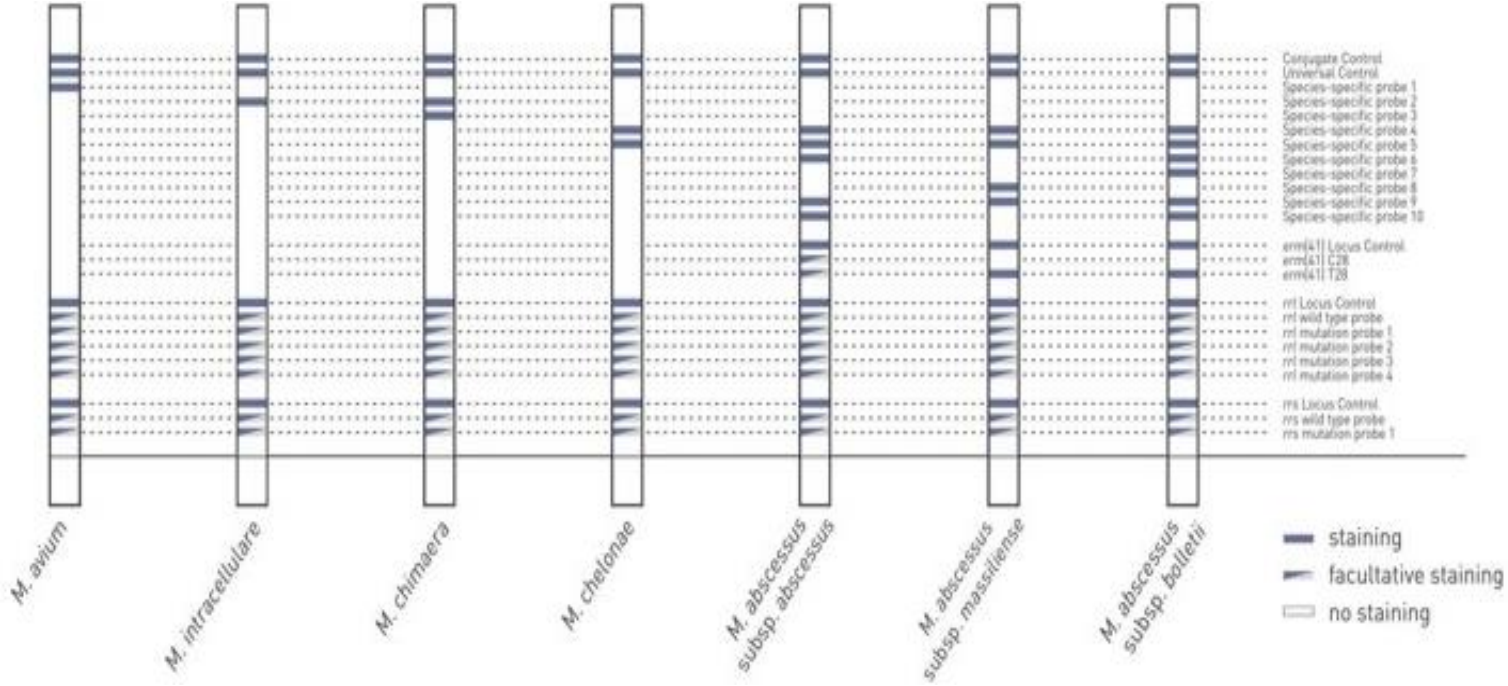
Differentiation of *M. tuberculosis* complex and different NTMs
- **GenoType Mycobacterium CM/AS (23S RNA gene)**

Differentiation of the *M. avium* and *M. abscessus* complex
- **GenoType NTM-DR**



Detection of mutations associated with AB resistance

- **GenoType NTM-DR:** resistance to macrolides and aminoglycosides



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Commercial kits

- GenoType NTM-DR:

GenoType NTM-DR			
Genes	**Probes**	**Mutations**	**Resistance**
erm(41)	C28	Only Interpretable for *M.absc ssp abscessus* and *bolletii.* *M.absc ssp massiliense* is deleted in erm(41) so always macrolides-sensitive except if rrl mutation .	Macrolides (clarithro ou azithro)-Sensitive
	T28		Macrolides (clarithro or azithro)-Resistant
rrl	MUT1	A2058C	Macrolides (clarithro or azithro)
	MUT2	A2058G	
	MUT3	A2059C	
	MUT4	A2059G	
	Delta WT	A2058T ou A2059T	
rrs	MUT1	A1408G	Aminoglycosides (kanamycin, amikacine, gentamicin)
	Delta WT	T1406A ou C1409T	

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Antibiotic Susceptibility test (AST)

Species identification

Partial report with remark:

The antibiogram will only be performed on request, if clinically relevant.


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ISO 15189 Accreditatie
Certificaat nr. 081-MED

Analyseverslag F22BD01668 Rapportdatum: 17/03/2022

Opmerkingen / interpretatie:	Een niet-tuberculeuze Mycobacterie geïsoleerd uit een respiratoir staal is niet noodzakelijk de oorzaak van een pathologie. Indien de klinische toestand van de patiënt dit vereist zullen wij op aanvraag een antibiogram uitvoeren.

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Antibiotic Susceptibility test (AST)

Proportion method of Canetti (solid medium)

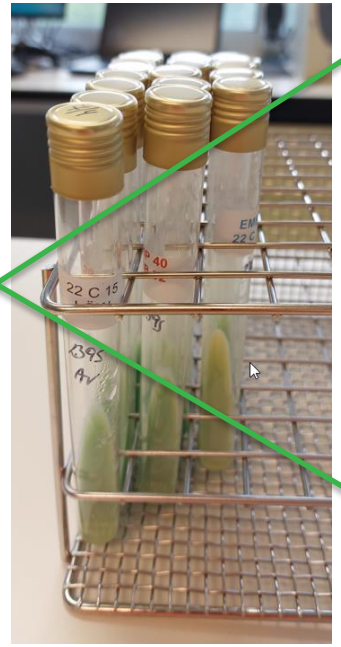
Revue de Tuberculose et de Pneumologie. T. **27**, 1963, n° 2-3 (pp. 217-272).

MESURE DE LA SENSIBILITÉ DU BACILLE TUBERCULEUX
AUX DROGUES ANTIBACILLAIRES
PAR LA MÉTHODE DES PROPORTIONS.

MÉTHODOLOGIE, CRITÈRES DE RÉSISTANCE,
RÉSULTATS, INTERPRÉTATION

par

G. CANETTI, N. RIST et J. GROSSET
(Institut Pasteur, Paris).



Broth microdilution method (Sensititre®)

From 15/04/2022, the Canetti technique for the determination of susceptibility to non-tuberculous mycobacteria will no longer be performed due to insufficient scientific evidence for interpretation. The microdilution method (Sensititre®) is recommended by the CLSI. If you have any questions, you can always contact us at BK@sciensano.be

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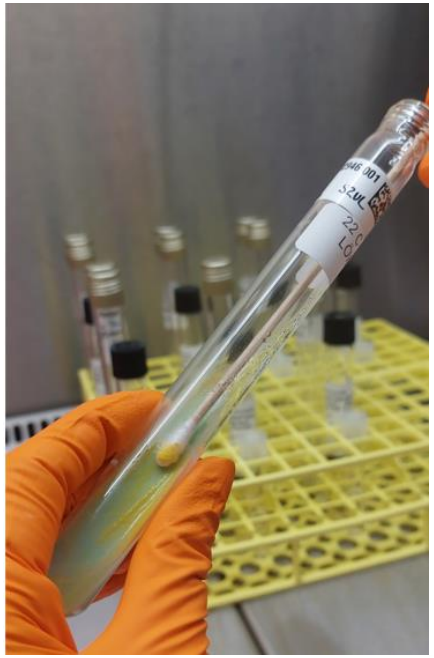
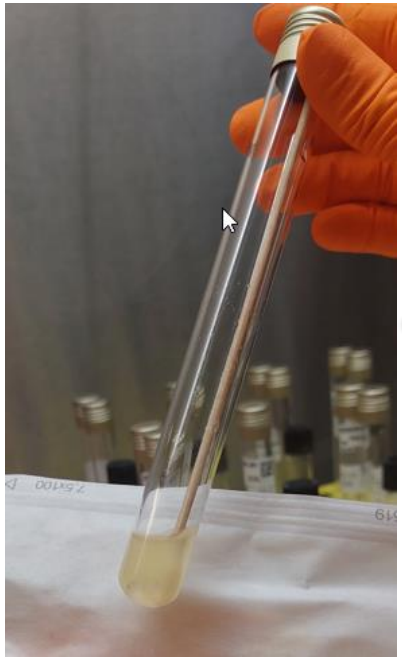
“Broth microdilution is the validated method for AST for NTM”

Antibiotic Susceptibility test (AST)

Preparation of the inoculum

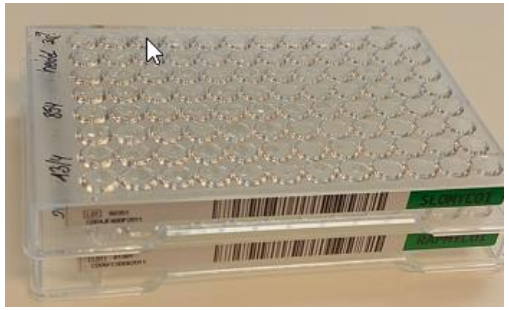


Scraping with a swab the slope of LJ tube



Suspension with glass beads

Adjust to 0.5-1 McFarland



Distribution in the plate

Incubation:

- **RGM: 4 to 14 days (inducible CLTM®)**
- **SGM: until the positive is readable**

Antibiotic Susceptibility test (AST)

Plate Code: **RAPMYCO**

	1	2	3	4	5	6	7	8	9	10	11	12
A	SXT 0.25/4.75	SXT 0.5/9.5	SXT 1/19	SXT 2/38	SXT 4/76	SXT 8/152	LZD 1	LZD 2	LZD 4	LZD 8	LZD 16	LZD 32
B	CIP 0.12	CIP 0.25	CIP 0.5	CIP 1	CIP 2	CIP 4	IMI 2	IMI 4	IMI 8	IMI 16	IMI 32	IMI 64
C	MXF 0.25	MXF 0.5	MXF 1	MXF 2	MXF 4	MXF 8	FEP 1	FEP 2	FEP 4	FEP 8	FEP 16	FEP 32
D	FOX 4	FOX 8	FOX 16	FOX 32	FOX 64	FOX 128	AUG2 2/1	AUG2 4/2	AUG2 8/4	AUG2 16/8	AUG2 32/16	AUG2 64/32
E	AMI 1	AMI 2	AMI 4	AMI 8	AMI 16	AMI 32	AMI 64	AXO 4	AXO 8	AXO 16	AXO 32	AXO 64
F	DOX 0.12	DOX 0.25	DOX 0.5	DOX 1	DOX 2	DOX 4	DOX 8	DOX 16	MIN 1	MIN 2	MIN 4	MIN 8
G	TGC 0.015	TGC 0.03	TGC 0.06	TGC 0.12	TGC 0.25	TGC 0.5	TGC 1	TGC 2	TGC 4	TOB 1	TOB 2	TOB 4
H	CLA 0.06	CLA 0.12	CLA 0.25	CLA 0.5	CLA 1	CLA 2	CLA 4	CLA 8	CLA 16	TOB 8	TOB 16	POS

ANTIMICROBICS

SXT	Trimethoprim / sulfamethoxazole
CIP	Ciprofloxacin
MXF	Moxifloxacin
FOX	Cefoxitin
AMI	Amikacin
DOX	Doxycycline
TGC	Tigecycline
CLA	Clarithromycin
LZD	Linezolid
IMI	Imipenem
FEP	Cefepime
AUG2	Amoxicillin / clavulanic acid 2:1 ratio
AXO	Ceftriaxone
MIN	Minocycline
TOB	Tobramycin
POS	Positive Control

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Antibiotic Susceptibility test (AST)

Plate Code: **SLOMYCOI**

	1	2	3	4	5	6	7	8	9	10	11	12
A	CLA 0.06	CLA 0.12	CLA 0.25	CLA 0.5	CLA 1	CLA 2	CLA 4	CLA 8	CIP 16	STR 64	DOX 16	ETH 20
B	CLA 16	CLA 32	CLA 64	MXF 8	RIF 8	SXT 8/152	AMI 64	LZD 64	CIP 8	STR 32	DOX 8	ETH 10
C	RFB 8	EMB 16	INH 8	MXF 4	RIF 4	SXT 4/76	AMI 32	LZD 32	CIP 4	STR 16	DOX 4	ETH 5
D	RFB 4	EMB 8	INH 4	MXF 2	RIF 2	SXT 2/38	AMI 16	LZD 16	CIP 2	STR 8	DOX 2	ETH 2.5
E	RFB 2	EMB 4	INH 2	MXF 1	RIF 1	SXT 1/19	AMI 8	LZD 8	CIP 1	STR 4	DOX 1	ETH 1.2
F	RFB 1	EMB 2	INH 1	MXF 0.5	RIF 0.5	SXT 0.5/9.5	AMI 4	LZD 4	CIP 0.5	STR 2	DOX 0.5	ETH 0.6
G	RFB 0.5	EMB 1	INH 0.5	MXF 0.25	RIF 0.25	SXT 0.25/4.75	AMI 2	LZD 2	CIP 0.25	STR 1	DOX 0.25	ETH 0.3
H	RFB 0.25	EMB 0.5	INH 0.25	MXF 0.12	RIF 0.12	SXT 0.12/2.38	AMI 1	LZD 1	CIP 0.12	STR 0.5	DOX 0.12	POS

ANTIMICROBICS

CLA	Clarithromycin
RFB	Rifabutin
EMB	Ethambutol
INH	Isoniazid
MXF	Moxifloxacin
RIF	Rifampin
SXT	Trimethoprim / sulfamethoxazole
AMI	Amikacin
LZD	Linezolid
CIP	Ciprofloxacin
STR	Streptomycin
DOX	Doxycycline
ETH	Ethionamide
POS	Positive Control

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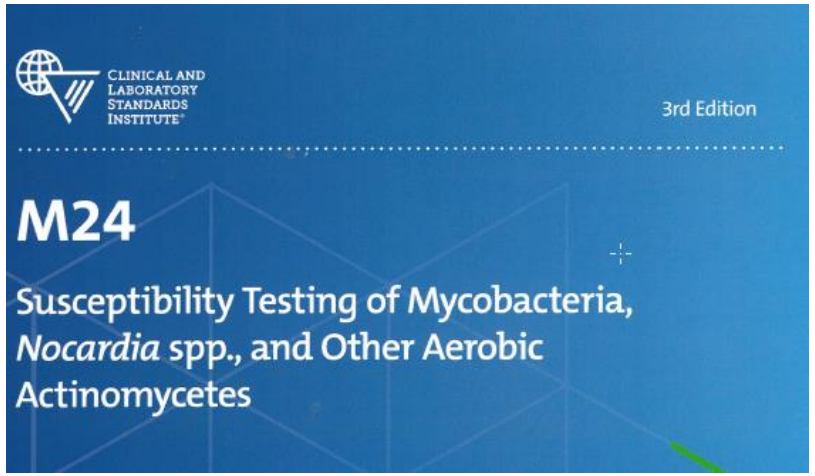
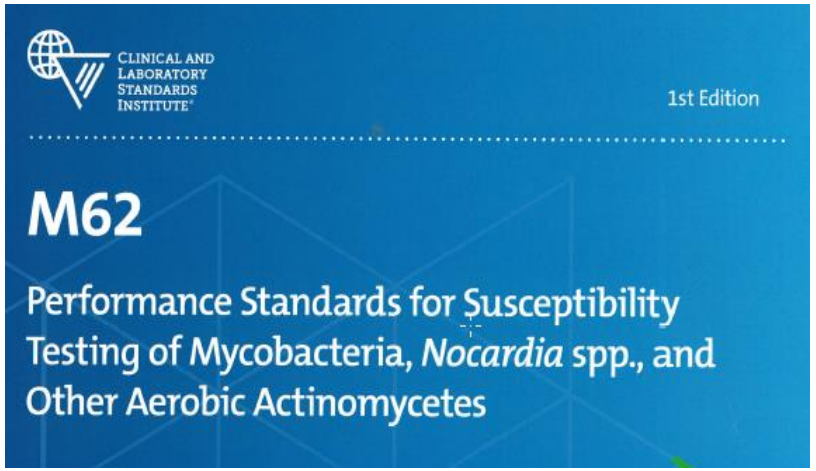
Antibiotic Susceptibility test (AST) Interpretation





			Sensitive	Intermediate	Resistant
Etude *in vitro* de la sensibilité aux antibiotiques par la technique Sensititre® (broth microdilution method for rapidly growing mycobacteria) - = Sensititre **RAPMYCO**	Linezolide	µg/ml	≤8	16	≥32
	Clarithromycine	µg/ml	≤2	4	≥8
	Amikacine	µg/ml	≤16	32	≥64
	Cefoxitine	µg/ml	≤16	32-64	≥128
	Ceftriaxone	µg/ml	NO CLSI value		
	Imipenem	µg/ml	≤4	8-16	≥32
	Tobramycine	µg/ml	≤2	4	≥8
	Ciprofloxacine	µg/ml	≤1	2	≥4
	Moxifloxacine	µg/ml	≤1	2	≥4
	Minocycline	µg/ml	NO CLSI value		
	Amoxicilline/acide Clavulanique	µg/ml			
	Trimethoprime/sulfamethoxazole	µg/ml	≤2/38	/	≥4/76
	Cefepime	µg/ml	No CLSI value		
	Tigecycline	µg/ml			
	Doxycycline	µg/ml	≤1	2-4	≥8
Etude *in vitro* de la sensibilité aux antibiotiques par la technique Sensititre® (broth microdilution method for slowly growing mycobacteria) = Sensititre **SLOMYCO kansasii**	Clarithromycine	µg/ml	≤8	16	≥32
	Rifampicine	µg/ml	≤1	/	≥2
	Amikacine	µg/ml	≤16	32	≥64
	Ciprofloxacine	µg/ml	≤1	2	≥4
	Ethambutol	µg/ml	No CLSI value		
	Linezolide	µg/ml	≤8	16	≥32
	Moxifloxacine	µg/ml	≤1	2	≥4
	Rifabutine	µg/ml	≤2	/	≥4
	Trimethoprime/sulfamethoxazole	µg/ml	≤2/38	/	≥4/76
	Doxycycline	µg/ml	≤1	2-4	≥8
	Isoniazide	µg/ml	No CLSI value		
	Streptomycine	µg/ml			
	Ethionamide	µg/ml			
Etude *in vitro* de la sensibilité aux antibiotiques par la technique Sensititre® (broth microdilution method for slowly growing mycobacteria) - = Sensititre **SLOMYCO MAC** (*Mycobacterium avium* complex)	Clarithromycine	µg/ml	≤8	16	≥32
	Rifampicine	µg/ml	No CLSI value		
	Amikacine (IV)	µg/ml	≤16	32	≥64
	Amikacine (liposomal inhaled)	µg/ml	≤64	-	≥128
	Ciprofloxacine	µg/ml	No CLSI value		
	Ethambutol	µg/ml			
	Linezolide	µg/ml	≤8	16	≥32
	Moxifloxacine	µg/ml	≤1	2	≥4
	Rifabutine	µg/ml	No CLSI value		
	Trimethoprime/sulfamethoxazole	µg/ml			
	Doxycycline	µg/ml			
	Isoniazide	µg/ml			
	Streptomycine	µg/ml			
	Ethionamide	µg/ml			
Etude *in vitro* de la sensibilité aux antibiotiques par la technique Sensititre® (broth microdilution method for slowly growing mycobacteria) - = Sensititre **SLOMYCO other than MAC and *kansasii***	Clarithromycine	µg/ml	≤8	16	≥32
	Rifampicine	µg/ml	≤1	/	≥2
	Amikacine	µg/ml	≤16	32	≥64
	Ciprofloxacine	µg/ml	≤1	2	≥4
	Ethambutol	µg/ml	No CLSI value		
	Linezolide	µg/ml	≤8	16	≥32
	Moxifloxacine	µg/ml	≤1	2	≥4
	Rifabutine	µg/ml	≤2	/	≥4
	Trimethoprime/sulfamethoxazole	µg/ml	≤2/38	/	≥4/76
	Doxycycline	µg/ml	≤1	2-4	≥8
	Isoniazide	µg/ml	No CLSI value		
	Streptomycine	µg/ml			
	Ethionamide	µg/ml			

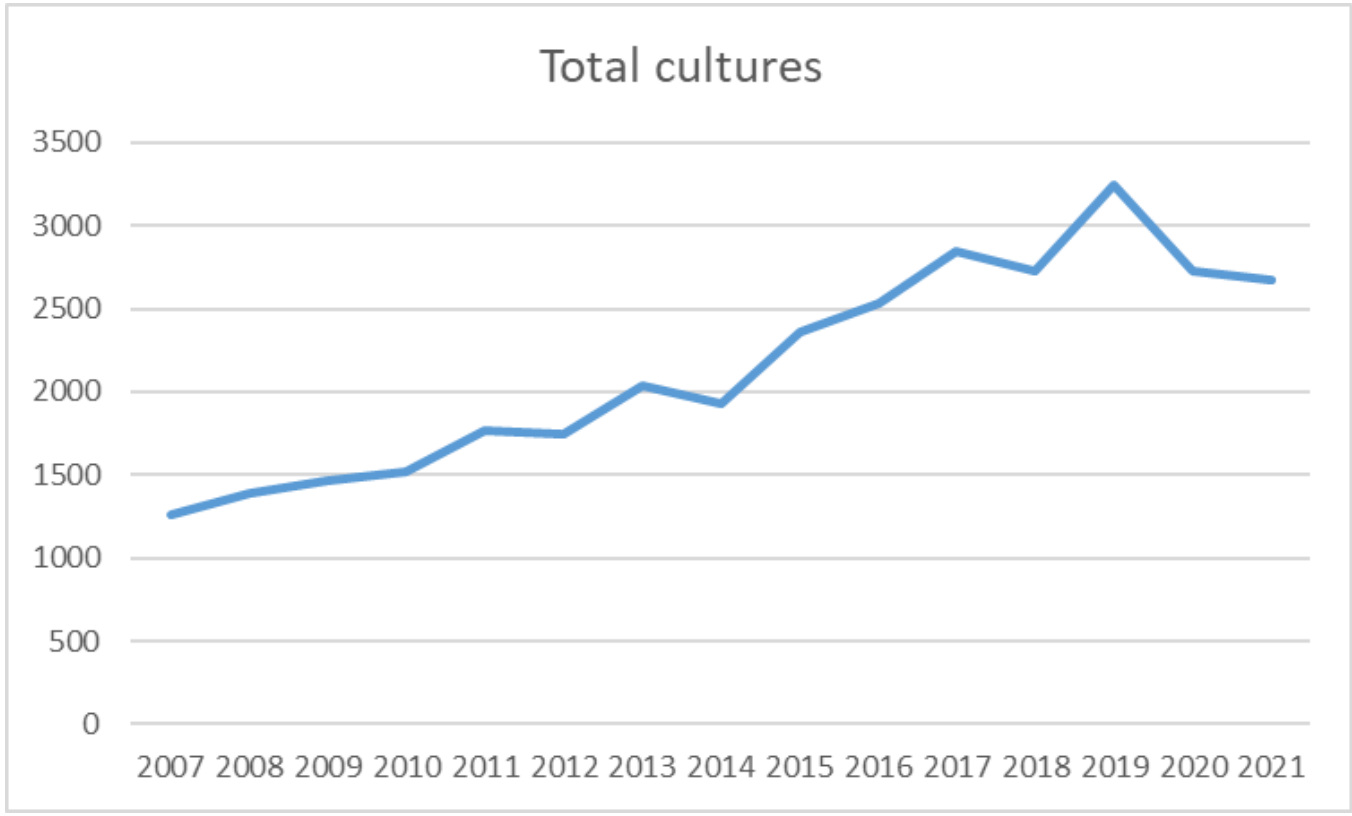
Epidemiology

Principal laboratory performing AST for clinically relevant cases

NTM surveillance/diagnosis data from the Belgian NRC since 2007

Evolution of the laboratory analysis

Year	Total cultures
2007	1265
2008	1390
2009	1462
2010	1517
2011	1765
2012	1743
2013	2042
2014	1929
2015	2366
2016	2534
2017	2851
2018	2729
2019	3243
2020	2725
2021	2674



Soetaert Karine, Subissi Lorenzo, Ceyssens Pieter-Jan, Vanfleteren Brigitte, Chantrenne Marianne, Asikainen Tommi, Duysburgh Els, Mathys Vanessa. Strong increase of true and false positive mycobacterial cultures sent to the National Reference Centre in Belgium, 2007 to 2016. Euro Surveill. 2019;24(11):pii=1800205. https://doi.org/10.2807/1560-7917.ES.2019.24.11.1800205

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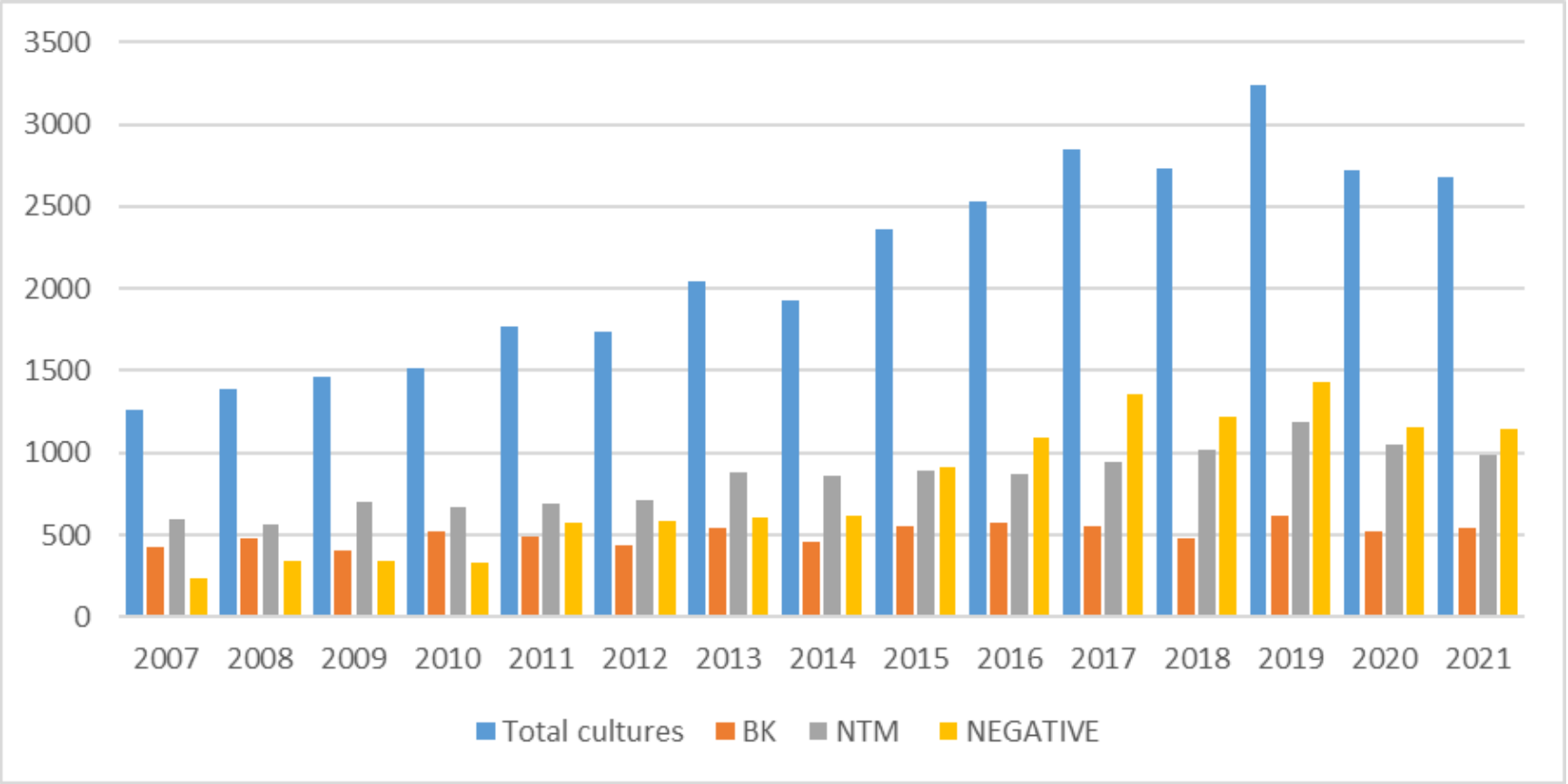
Evolution of the laboratory analysis



Increasing numbers of NTM

- improved culturing technique
- increased awareness
- increase in disease incidence

Soetaert Karine, Subissi Lorenzo, Ceyssens Pieter-Jan, Vanfleteren Brigitte, Chantrenne Marianne, Asikainen Tommi, Duysburgh Els, Mathys Vanessa. Strong increase of true and false positive mycobacterial cultures sent to the National Reference Centre in Belgium, 2007 to 2016. Euro Surveill. 2019;24(11):pii=1800205. https://doi.org/10.2807/1560-7917.ES.2019.24.11.1800205

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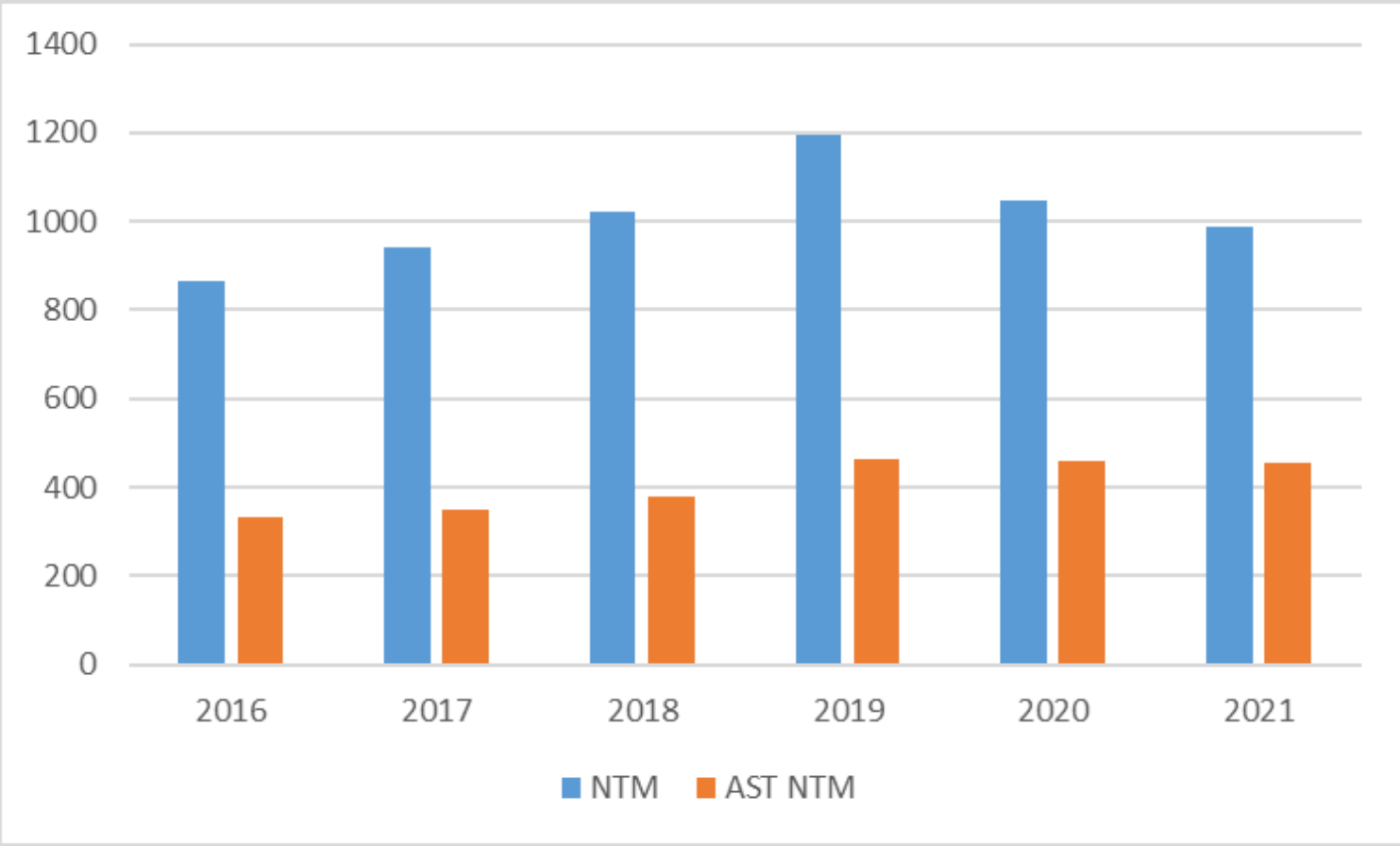
Evolution of the laboratory analysis

Year	NTM	AST NTM
2016	867	334
2017	941	351
2018	1022	381
2019	1193	462
2020	1046	459
2021	988	456



AST on NTM species is only performed upon physicians' request.

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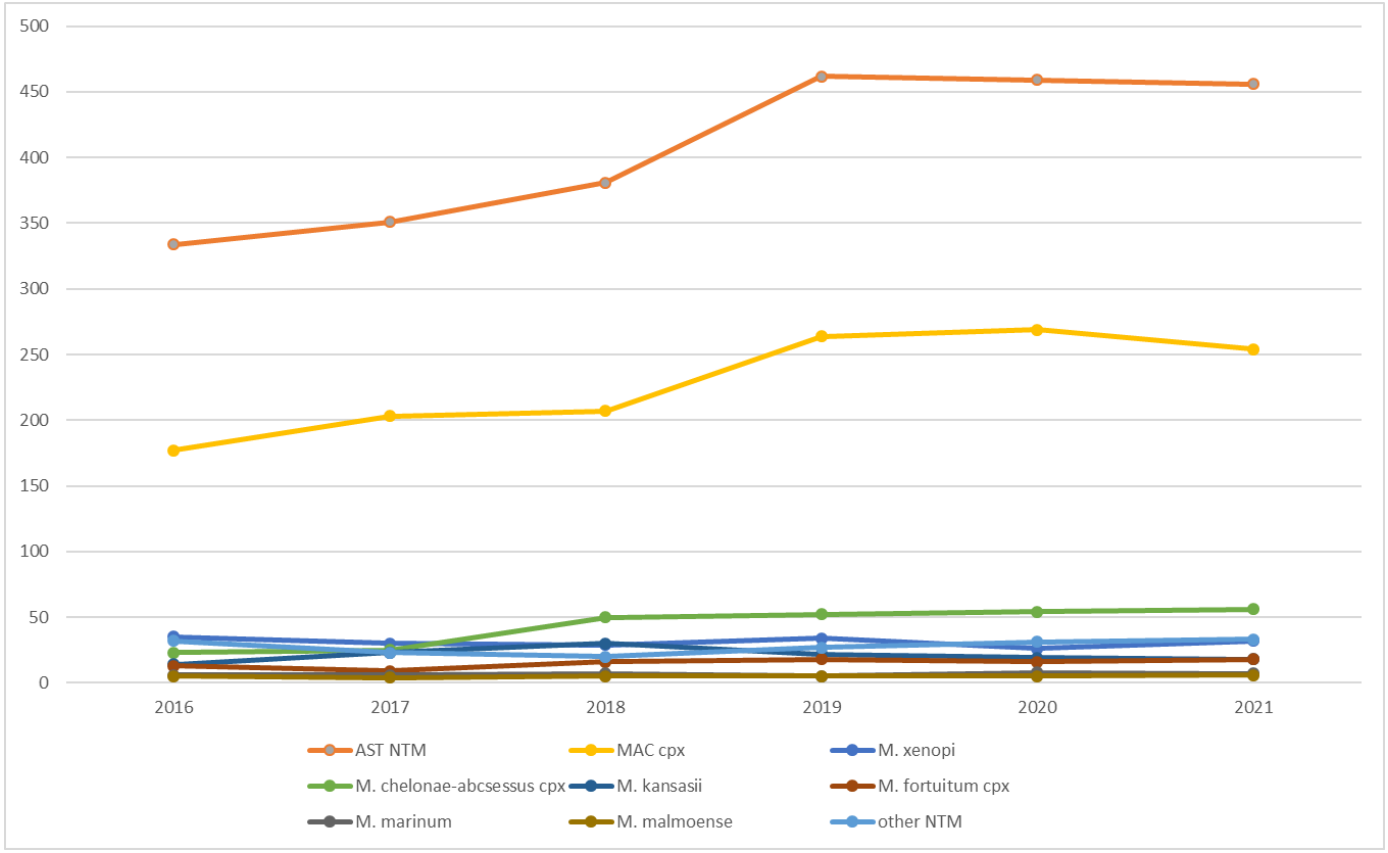
Evolution of the laboratory analysis



The correct identification of the aetiological agent is critical for diagnosis and patient management, as species differ in clinical relevance, antibiotic susceptibility and treatment outcome

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Thank you for your attention

NRC Mycobacterium

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