

CAT: Identificatie en gevoeligheidsbepaling van Nocardia

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Kweek en identificatietechnieken: rol van MALDI-TOF MS?

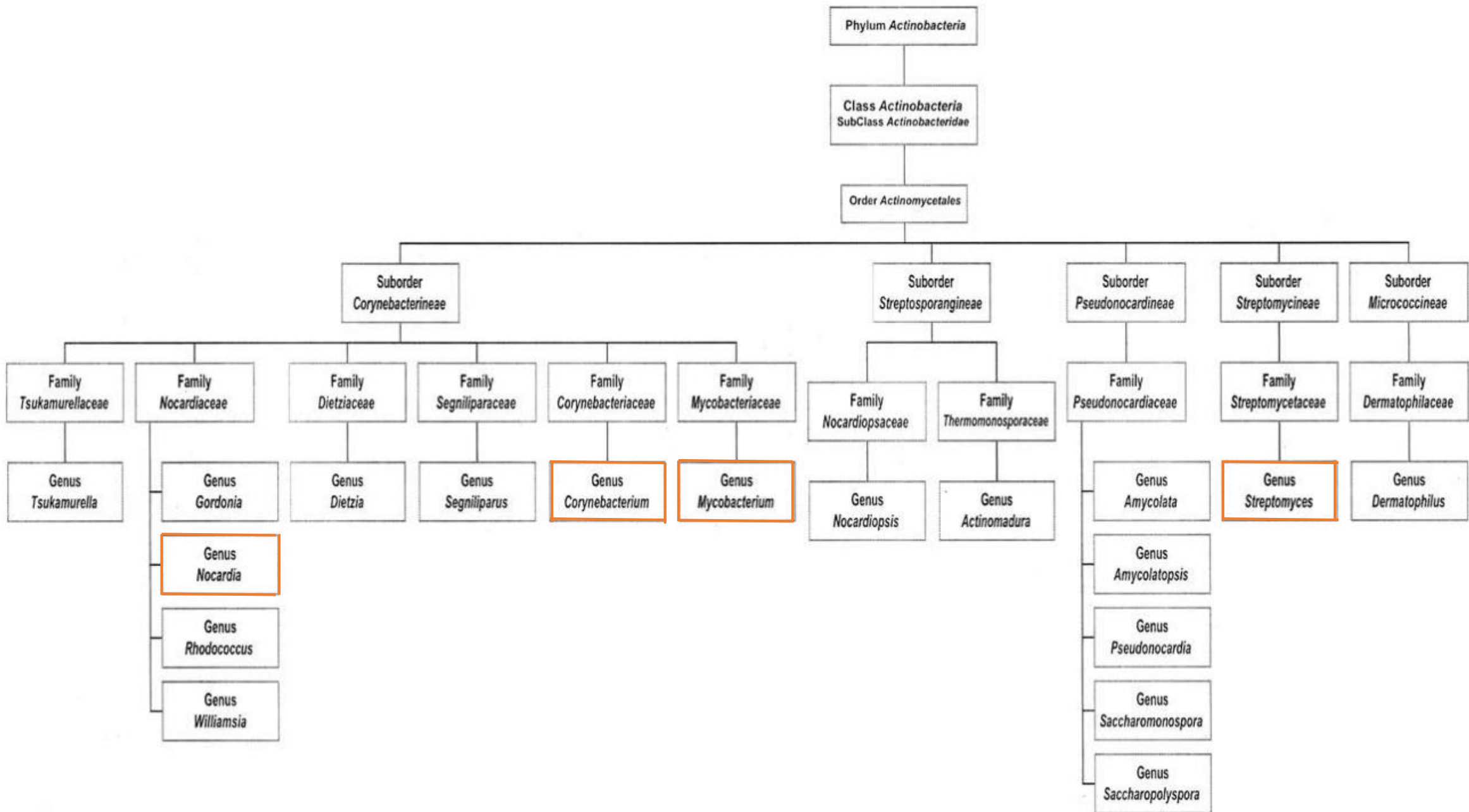
Gevoeligheidsbepaling: Etest versus BMD?

Gevoeligheidsbepaling: meest geschikte methode voor UZ Leuven?

Historiek

- eerste beschrijving door Franse dierenarts
- Edmond Nocard in 1888
- runderen met chronische lymfadenitis





Algemeen

- geen deel van humane flora
- saprophyten in bodem, rottende organische materie & water
- incidentie: 0,45/100.000 inwoners/jaar in Spanje
- inhalatie = voornaamste route van besmetting
- 2-3♂/ 1♀

Taxonomie

Nocardia species

voor 1988

N. asteroides > 90%

1988
Wallace

TABLE 1. Drug patterns and their major group characteristics among 78 clinical isolates of *N. asteroides*

Drug pattern type	Incidence (%)	Major group characteristics
1	20	Susceptible to ampicillin, carbenicillin, and broad-spectrum cephalosporins; imipenem MICs for one-half of isolates were high (8–32 µg/ml)
2	0	Same as type 1 except that kanamycin MICs were low (<1 µg/ml); susceptible to ciprofloxacin
3	18	Susceptible to ampicillin but resistant to carbenicillin; susceptible to erythromycin
4	5	Resistant all aminoglycosides, including amikacin; susceptible to ciprofloxacin
5	17	Resistant to penicillins and broad-spectrum cephalosporins; resistant to all aminoglycosides except amikacin; susceptible to ciprofloxacin and imipenem
6	35	Resistant to penicillins but susceptible to broad-spectrum cephalosporins
Miscellaneous	5	Unable to group with above

Corresponding type drug pattern	Major drug pattern characteristics
I	Susceptible to ampicillin, amoxicillin-clavulanic acid, ceftriaxone, linezolid, and amikacin; most have resistant MICs for imipenem; resistant to ciprofloxacin and clarithromycin
II	Same as type I but kanamycin MICs low (<1 µg/ml) and susceptible to ciprofloxacin; usually resistant to gentamicin; resistant to clarithromycin
III	Susceptible to ampicillin but resistant to amoxicillin-clavulanic acid; susceptible to erythromycin, clarithromycin, linezolid, and ceftriaxone; very low MICs to imipenem and amikacin
IV	Resistant to all aminoglycosides, including amikacin; susceptible to ciprofloxacin, ceftriaxone, linezolid, and imipenem; resistant to erythromycin and clarithromycin
V	Resistant to ampicillin, broad-spectrum cephalosporins, and clarithromycin; resistant to aminoglycosides except amikacin; susceptible to ciprofloxacin, linezolid, and imipenem
VI	Resistant to ampicillin, amoxicillin-clavulanic acid, clarithromycin, and ciprofloxacin; susceptible to ceftriaxone, amikacin, linezolid, and imipenem

N. asteroides complex

Taxonomie

Nocardia species

heden

gevoeligheidspatroon*

type I

type III

type IV

type II

/
type VI
type V

Complex	Basis for complexing	Species Included In the complex
<i>N. abscessus</i> complex	MALDI-TOF MS profile Gene sequence (500 bp of 16S rRNA)	<i>N. abscessus</i> , <i>N. arthritidis</i> , <i>N. asiatica</i> , <i>N. beijingensis</i> , <i>N. pneumoniae</i> <i>N. abscessus</i> , <i>N. arthritidis</i> , <i>N. asiatica</i> , <i>N. beijingensis</i>
<i>N. nova</i> complex	Gene sequences (16S rRNA and/or <i>secA1</i>) Antibiotic susceptibility pattern MALDI-TOF MS profile	<i>N. africana</i> , <i>N. aobensis</i> , <i>N. cerradoensis</i> , <i>N. elegans</i> , <i>N. kruczakiae</i> , <i>N. mikamii</i> , <i>N. nova</i> , <i>N. vermiculata</i> , <i>N. veterana</i> <i>N. africana</i> , <i>N. elegans</i> , <i>N. kruczakiae</i> , <i>N. nova</i> , <i>N. veterana</i> <i>N. africana</i> , <i>N. aobensis</i> , <i>N. elegans</i> , <i>N. kruczakiae</i> , <i>N. nova</i> , <i>N. veterana</i>
<i>N. transvalensis</i> complex	Antibiotic susceptibility pattern Gene sequences (16S rRNA, <i>secA1</i>) MALDI-TOF MS profile	<i>N. blacklockiae</i> , <i>N. transvalensis</i> , <i>N. wallacei</i>
<i>N. brevicatena</i> / <i>N. paucivorans</i> complex	Antibiotic susceptibility pattern Gene sequences (16S rRNA, <i>secA1</i>) MALDI-TOF MS profile	<i>N. brevicatena</i> , <i>N. paucivorans</i>
<i>N. otitidiscaviarum</i> complex	Gene sequences (16S rRNA, <i>hsp65</i>)	Various strains of <i>N. otitidiscaviarum</i>
<i>N. cyriacigeorgica</i> complex	16S rRNA gene sequence	Various strains of <i>N. cyriacigeorgica</i>
<i>N. farcinica</i> complex	Gene sequences (16S rRNA, <i>secA1</i>)	<i>N. farcinica</i> , <i>N. kroppenstedtii</i>

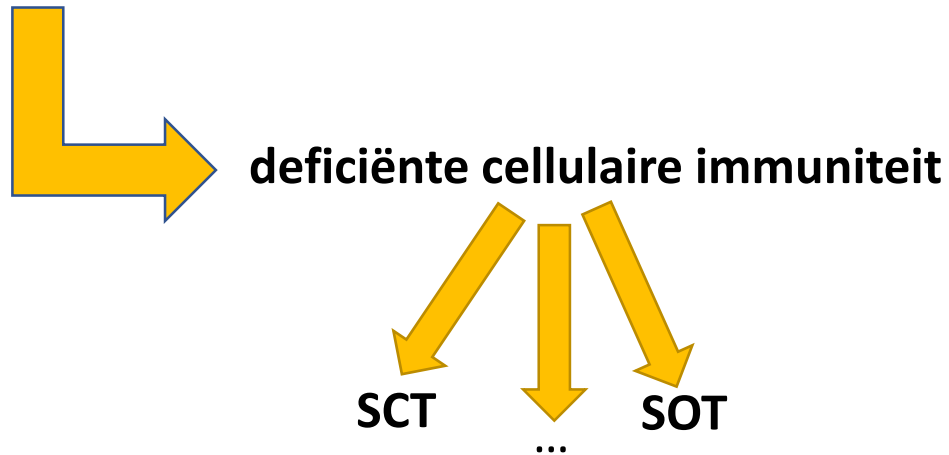
...

Taxonomie

Species	Year described	significance ^b	Primary skin	Pulmonary	Disseminated	source				
<i>N. abscessus</i>	2000			+	+		<i>N. kruczakiae</i>	2004	+	
<i>N. africana</i>	2001			+			<i>N. mexicana</i>	2006		+
<i>N. amikacinitorans</i>	2012	+ ^c		+			<i>N. mikamii</i>	2010		+
<i>N. anaemiae</i>	2005	+					<i>N. niigatensis</i>	2004		+
<i>N. aobensis</i>	2005	+				+	<i>N. ninae</i>	2007	+	
<i>N. araoensis</i>	2004	+					<i>N. niwae</i>	2011		+
<i>N. arthritis</i>	2004	+					<i>N. nova</i>	1983		+
<i>N. asiatica</i>	2004			+			<i>N. otitidiscaviarum</i>	1924		+
<i>N. asteroides</i>	1891	+					<i>N. paucivorans</i>	2000	+	
<i>N. beijingensis</i>	2001			+	+		<i>N. pneumoniae</i>	2004	+	
<i>N. blacklockiae</i>	2008	+					<i>N. pseudobrasiliensis</i>	1995		+
<i>N. brasiliensis</i>	1909		+				<i>N. puris</i>	2003	+	
<i>N. brevicatena</i>	1961	+					<i>N. sienata</i>	2004	+	
<i>N. carnea</i>	1891	+					<i>N. terpenica</i>	2007	+	
<i>N. concava</i>	2005	+					<i>N. testaceae</i>	2004	+	
<i>N. cyriaciageorgica</i>	2001			+	+		<i>N. thailandica</i>	2005	+	
<i>N. elegans</i>	2005	+					<i>N. transvalensis</i>	1927		+
<i>N. exalbida</i>	2006	+ ^c					<i>N. vermiculata</i>	2005	+	
<i>N. farcinica</i>	1889			+	+		<i>N. veterana</i>	2001		+
<i>N. higoensis</i>	2004			+	+		<i>N. wallacei</i>	2006		+
<i>N. inohanensis</i>	2004	+					<i>N. yamanashiensis</i>	2004	+	

Kliniek

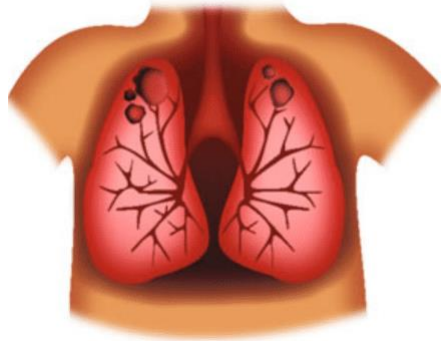
- **opportunistische** pathogeen doch 1/3 normale afweer



risicofactoren bij orgaantransplantaties

- hoge dosis **corticosteroiden** gebruik (≥ 20 mg of prednisone voor ≥ 1 maand)
- **CMV-infectie** in voorafgaande zes maanden
- **hoge [calcineurineremmers]** in voorafgaande maand ($> 15 \mu\text{g/ml}$ voor tacrolimus of $> 300 \text{ ng/ml}$ voor cyclosporine)

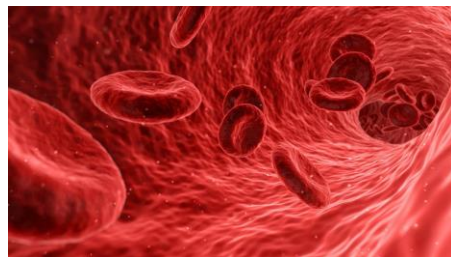
Kliniek



- **frequentste manifestatie**
- subacuut tot chronisch verloop
- aspecifieke symptomatologie
- beeldvorming : divers
- differentiaal diagnose
 - *P. aeruginosa*, *S. aureus*, anaëroben
 - IPA, actinomyose, tuberculose
 - maligniteit
 - ...



- **frequentste extrapulmonale** manifestatie
- verscheidenheid aan symptomen
- beeldvorming meestal ≥ 1 **hersenenabces**



- **minder frequent**
- associatie met aanwezigheid DVC en/of verminderde afweer



- **ook bij normaal immuunsysteem**
- primaire of secundaire infectie
- ulcera, abcessen, noduli, cellulitis
- voornaamste verwekker: ***N. brasiliensis***
- **typisch in (sub-)tropisch klimaat**

Microscopisch onderzoek

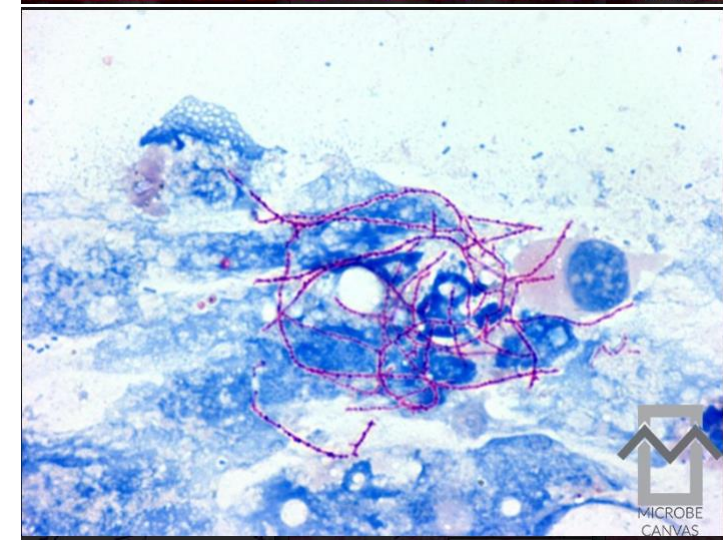
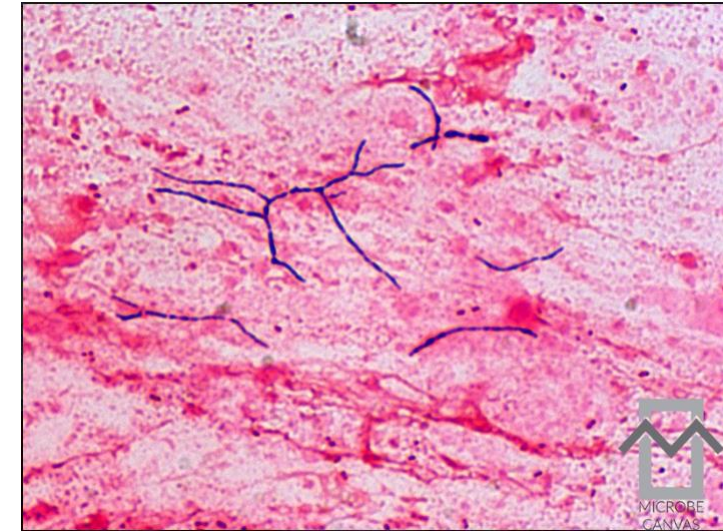


- gramkleuring  zuurvaste kleuring

- sensitiviteit gram > sensitiviteit zuurvast

- = variant Kinyounmethode (1% H_2SO_4)
 - belang van + en - controles!
 - snelle differentiatie Nocardia en actinomyces

	Actinomyces	Mycobacterium	Nocardia	Streptomyces
Ziehl-Neelsen kleuring	-	+	-	-
variant Kinyoun kleuring	-	+	+	-



Isolatieprocedure

- Groei op klassieke niet-selectieve voedingsbodems
- Typische kolonievorming na 2-5 dagen incubatie

steriel staal	niet-steriel staal
bloedagar + THIO + TSB	cfr. steriel + BCYE

- Afsluiting platen d.m.v. parafilm & incuberen gedurende **3 weken bij 35°C**
- Aflezing eerste week dagelijks, nadien wekelijks

Identificatie

- belang van snelle en accurate identificatie tot op species niveau! (cfr. later)
- biochemische identificaties: tijdrovend en grotendeels onbetrouwbaar
- MALDI-TOF MS ???
- **moleculaire** techniek = **gouden standaard**
 - sequentieanalyse 16S-rRNA gen, secA1 gen, hsp65 gen en/of gyrB gen

Vraag 1: Kan MALDI-TOF MS gebruikt worden voor de identificatie van Nocardia species?

- Opzet: literatuurstudie: MALDI-TOF MS (8 artikels)
- Referentiemethode: gen sequentieanalyse
- CLSI criterium: $\geq 90\%$ overeenkomst tussen referentie- en nieuwe methode
- UZ Leuven: MALDI-TOF MS **BDAL-8468**



studie en jaar publicatie databank (Nocardia spectra) ± in huis databank (Nocardia spectra)	aantal stammen	% correcte identificatie		incubatietijd	extractieprocedure
		species (cutoff)	groep (cutoff)		
Verroken et al. 2010 BDAL 3486 (v.3.0) (X) + in-huis databank (110)	43	23 (≥ 2.0) 79 (≥ 2.0)	44 (≥ 1.7) 88 (≥ 1.7)	48-72u	EtOH/FA
Hsueh et al. 2014 BDAL 5627 (72)	74	14,9 (≥ 2.0)	70,1 (≥ 1.7)	48u	EtOH/FA
Buckwalter et al. 2015 BDAL 5627 v.3.3.1.0 (72) + in-huis databank (232)	148	41,9 (≥ 2.0) 89,9 (≥ 2.0)	57,4 (≥ 1.7) 94,6 (≥ 1.7)	onbekend	EtOH/FA
Khot et al. 2015 BDAL 5627 v.3.1 (72) + in-huis databank (13)	87	53 (≥ 1.9) 83,1 (≥ 1.9)	62 (≥ 1.7) 94,8 (≥ 1.7)	18-48u	EtOH/FA
Blosser et al. 2016 BDAL 5627 v.4.0.0.1 (72) + in-huis databank (90)	150	47,3 (≥ 2.0) 84,2 (≥ 2.0)	72 (≥ 1.8) 90,2 (≥ 1.8)	72-96u	EtOH/FA
Yarbrough et al. 2017 BDAL 5989 v.5 (73) BDAL 6903 v.6 (105)	60	43 (≥ 2.0) 58 (≥ 2.0)	77 (≥ 1.7) 83 (≥ 1.7)	48-144u	EtOH/FA
Marín et al. 2018 BDAL 6903 (v.6) (105)	73	57,5 (≥ 2.0) 94,5 (≥ 1.7)	95,9 (≥ 1.7) 98,6 (≥ 1.5)	36-48u	directe applicatie mierenzuur
McTaggart et al. 2018 BDAL 6903 (v.6) (105) + in-huis databank (X)	251	80,9 (≥ 2.0) 87,3 (≥ 2.0)	86,9 (≥ 1.7) 93,3 (≥ 1.7)	18-72u	EtOH/FA

Effect van verlaging scoredrempel

- Acceptatiecriteria Bruker
 - score voor ID genus $\geq 1,7$
 - score voor ID species $\geq 2,0$
- Quid effect verlaging score voor species niveau naar $\geq 1,8$?

Center	% isolates with the following score threshold/margin ^a :	
	≥ 2.0 cutoff/10% rule	≥ 1.8 cutoff/10% rule
1	83.3	89.3
2	89.3	92.6
3	80.0	87.3
Combined	84.2	89.8

Invloed van leeftijd cultuur (1a)

TABLE 2 Comparison of MALDI-TOF MS identifications when isolates were tested at <48 h and >48 h

Organism	MALDI-TOF MS ID at <48 h (reported result ^a)	Score at <48 h ^b	MALDI-TOF MS ID at >48 h (reported result ^a)	Score at >48 h ^b
<i>Nocardia abscessus</i> complex	<i>Nocardia asiatica</i>	1.968	<i>Nocardia asiatica</i> (no ID)	1.663
<i>Nocardia abscessus</i> complex	<i>Nocardia asiatica</i>	2.229	<i>Nocardia asiatica</i>	2.101
<i>Nocardia aobensis</i>	<i>Nocardia aobensis</i> (<i>Nocardia</i> sp.)	1.825	<i>Nocardia aobensis</i> (<i>Nocardia</i> sp.)	1.82
<i>Nocardia beijingensis</i>	<i>Nocardia araoensis</i> (<i>Nocardia</i> sp.)	1.791	<i>Pseudomonas jinjuensis</i> (no ID)	1.448
<i>Nocardia brasiliensis</i>	<i>Nocardia brasiliensis</i>	2.37	<i>Nocardia brasiliensis</i>	2.409
<i>Nocardia carnea</i>	<i>Nocardia asiatica</i> (no ID)	1.574	<i>Nocardia farcinica</i> (no ID)	1.365
<i>Nocardia cyriacigeorgica</i>	<i>Nocardia cyriacigeorgica</i>	2.385	<i>Nocardia farcinica</i> (no ID)	1.414
<i>Nocardia cyriacigeorgica</i>	<i>Nocardia cyriacigeorgica</i>	1.945	<i>Nocardia cyriacigeorgica</i> (no ID)	1.558
<i>Nocardia cyriacigeorgica</i>	<i>Nocardia cyriacigeorgica</i>	1.922	<i>Nocardia brasiliensis</i> (no ID)	1.245
<i>Nocardia cyriacigeorgica</i>	<i>Nocardia cyriacigeorgica</i> (<i>Nocardia</i> sp.)	1.811	<i>Streptococcus agalactiae</i> (no ID)	1.246
<i>Nocardia cyriacigeorgica</i>	<i>Nocardia cyriacigeorgica</i>	2.362	<i>Nocardia cyriacigeorgica</i> (no ID)	1.447
<i>Nocardia cyriacigeorgica</i>	<i>Nocardia cyriacigeorgica</i> (<i>Nocardia</i> sp.)	1.866	<i>Nocardia farcinica</i> (no ID)	1.41
<i>Nocardia cyriacigeorgica</i>	<i>Nocardia cyriacigeorgica</i> (<i>Nocardia</i> sp.)	1.853	<i>Salmonella</i> sp (no ID)	1.164
<i>Nocardia farcinica</i>	<i>Nocardia farcinica</i>	2.233	<i>Nocardia farcinica</i> (<i>Nocardia</i> sp.)	1.713
<i>Nocardia farcinica</i>	<i>Nocardia farcinica</i>	2.239	<i>Nocardia farcinica</i> (no ID)	1.578
<i>Nocardia farcinica</i>	<i>Nocardia farcinica</i>	2.292	<i>Nocardia farcinica</i> (<i>Nocardia</i> sp.)	1.833
<i>Nocardia ignorata</i>	<i>Nocardia asteroides</i> (no ID)	1.563	<i>Nocardia asteroides</i> (no ID)	1.599
<i>Nocardia nova</i>	<i>Nocardia nova</i>	2.217	<i>Nocardia nova</i>	2.375

Invloed van leeftijd cultuur (1b)

<i>Nocardia nova</i>	<i>Nocardia veterana</i> (<i>Nocardia</i> sp.)	1.736	<i>Nocardia veterana</i> (no ID)	1.494
<i>Nocardia nova</i>	<i>Nocardia nova</i>	2.009	<i>Nocardia nova</i>	2.014
<i>Nocardia nova</i>	<i>Nocardia nova</i>	2.105	<i>Nocardia nova</i>	2.108
<i>Nocardia nova</i>	<i>Nocardia nova</i>	2.199	<i>Nocardia nova</i>	2.023
<i>Nocardia nova</i>	<i>Nocardia nova</i>	2.092	<i>Nocardia nova</i>	1.954
<i>Nocardia nova</i>	<i>Nocardia nova</i>	2.028	<i>Nocardia nova</i> (no ID)	1.631
<i>Nocardia paucivorans</i>	<i>Nocardia paucivorans</i>	2.488	<i>Nocardia paucivorans</i>	2.521
<i>Nocardia pseudobrasiliensis</i>	<i>Nocardia pseudobrasiliensis</i>	1.915	<i>Nocardia pseudobrasiliensis</i> (no ID)	1.628
<i>Nocardia pseudobrasiliensis</i>	<i>Nocardia pseudobrasiliensis</i>	2.122	<i>Nocardia pseudobrasiliensis</i> (<i>Nocardia</i> sp.)	1.864
<i>Nocardia pseudobrasiliensis</i>	<i>Nocardia pseudobrasiliensis</i>	2.245	<i>Nocardia pseudobrasiliensis</i>	1.907
<i>Nocardia puris</i>	<i>Nocardia puris</i>	2.362	<i>Nocardia puris</i>	2.352
<i>Nocardia puris</i>	<i>Nocardia puris</i>	2.527	<i>Nocardia puris</i>	2.386
<i>Nocardia testacea</i>	<i>Nocardia testacea</i> (<i>Nocardia</i> sp.)	1.741	<i>Nocardia puris</i> (no ID)	1.558
<i>Nocardia transvalensis</i> complex	<i>Nocardia veterana</i> (no ID)	1.643	<i>Nocardia farcinica</i> (no ID)	1.35
<i>Nocardia transvalensis</i> complex	<i>Nocardia wallacei</i>	2.152	<i>Nocardia wallacei</i> (no ID)	1.393
<i>Nocardia transvalensis</i> complex	<i>Nocardia wallacei</i>	2.699	<i>Nocardia wallacei</i> (<i>Nocardia</i> sp.)	1.852
<i>Nocardia transvalensis</i> complex	<i>Nocardia wallacei</i>	2.515	<i>Nocardia wallacei</i>	2.433
<i>Nocardia vinacea</i>	<i>Nocardia vinacea</i>	2.172	<i>Nocardia vinacea</i>	2.321
Overall avg		2.083		1.769

^a If different from MALDI-TOF MS identification (ID).

^b Isolates with scores of ≥ 1.7 but < 1.9 were identified only to the genus level. Isolates with scores of < 1.7 were considered unidentified (no ID).

Invloed van leeftijd cultuur (2a)

Number and percentage of *Nocardia* isolates correctly identified by MALDI-ToF MS as a single species with a score ≥ 2.00 .

Culture conditions MALDI-ToF MS preparation	"Standard time" method		"Early growth" method			
	SAB ₃ 3 d at 35 °C		CBA, 35 °C until growth observed			
	EtOH/FA extraction + bead beating, Bruker library v6		EtOH/FA extraction, Bruker library v6		+ in-house library entries	+ bead beating
	No. isolates	No. (%) correct ID	No. isolates	No. (%) correct ID		Total
Common species¹						
<i>N. abscessus</i>	21	4 (19.0%)	21	18 (85.7%)	0 (0%) ²	19 (90.5%)
<i>N. brasiliensis</i>	9 ³	0 (0%)	9	3 (33%)	6 (66.7%)	9 (100%)
<i>N. cyriacigeorgica</i>	64 ³	32 (50.0%)	63	61 (96.8%)	n/a ⁴	62 (98.4%)
<i>N. farcinica</i> ⁵	41	18 (43.9%)	44	44 (100%)	n/a	44 (100%)
<i>N. nova complex</i> ⁶	55	25 (45.5%)	52	44 (84.6%)	n/a	47 (90.4%)
<i>N. otitidiscaviarum</i>	15	4 (26.7%)	13	12 (92.3%)	n/a	13 (100%)
Total common species	205	83 (40.5%)	202	182 (90.0%)	6 (3.0%)	194 (96.0%)
Rare species						
<i>N. asiatica</i>	7	3 (42.9%)	7	2 (28.6%)	4 (57.1%)	6 (85.7%)
<i>N. anaemiae</i>	1	1 (100%)	1	1 (100%)	n/a	1 (100%)
<i>N. arthritidis cluster</i>	8	0 (0%)	7	0 (0%)	6 (85.7%)	6 (85.7%)
<i>N. asteroides sensu stricto</i>	3	2 (66.7%)	3 ³	1 (33%)	n/a	1 (33%)
<i>N. beijingensis</i>	3	0 (0%)	2 ³	0 (0%)	n/a	0 (0%)
<i>N. cavia</i>	1	0 (0%)	1	1 (100%)	n/a	1 (100%)
<i>N. couleae/ignorata cluster</i>	3	0 (0%)	3	0 (0%)	n/a	0 (0%)
<i>N. exalbida</i>	1	0 (0%)	1	0 (0%)	n/a	0 (0%)
<i>N. neocaledoniensis</i>	2	0 (0%)	2	0 (0%)	n/a	0 (0%)
<i>N. paucivorans</i>	1	1 (100%)	1	1 (100%)	n/a	1 (100%)
<i>N. pneumoniae</i>	4	0 (0%)	5	4 (80%)	n/a	4 (80%)
<i>N. pseudobrasiliensis</i>	1	0 (0%)	1	1 (100%)	n/a	1 (100%)
<i>N. sientata</i>	2	1 (50%)	2	1 (50%)	n/a	1 (50%)
<i>N. thailandica</i>	2	0 (0%)	2	0 (0%)	n/a	0 (0%)
<i>N. transvalensis</i>	2	0 (0%)	2	1 (50%)	n/a	1 (50%)
<i>N. veterana</i>	2	2 (100%)	2	2 (100%)	n/a	2 (100%)
<i>N. wallacei</i>	7	0 (0%)	7	6 (85.7%)	n/a	6 (85.7%)
Total rare species	50	10 (20.0%)	49	21 (42.9%)	10 (20.4%)	31 (63.3%)
Grand Total	255	94 (36.9%)	251	203 (80.9%)	16 (6.4%)	225 (89.6%)

Invloed van leeftijd cultuur (2b)

Comparison of media (SAB and CBA), incubation time (18, 24, 48, 72h) and EtOH/FA extraction with and without bead-beating on MALDI-ToF identification scores of 10 isolates of *Nocardia*. Results coded: underlined ≥ 2.00 , italicized < 1.70 .

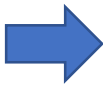
Molecular ID	18 h		24 h		48 h		72 h	
	EtOH/FA	Beads+ EtOH/FA	EtOH/FA	Beads+ EtOH/FA	EtOH/FA	Beads+ EtOH/FA	EtOH/FA	Beads+ EtOH/FA
<u>SAB at 35 °C</u>								
<i>N. abscessus</i>	n/a ¹	n/a	ng ²	ng	1.70	<i><1.70</i>	1.89	1.97
<i>N. abscessus</i>	n/a	n/a	ng	ng	1.76	<u>2.13</u>	1.82	1.89
<i>N. cyriacigeorgica</i>	<u>2.38</u>	<u>2.42</u>	<u>2.44</u>	<u>2.27</u>	<u>2.23</u>	<u>2.27</u>	<i><1.70</i>	<i><1.70</i>
<i>N. cyriacigeorgica</i>	<u>1.76</u>	<u>2.05</u>	<u>1.94</u>	<u>1.98</u>	<u>1.74</u>	<u>1.73</u>	1.84	1.74
<i>N. cyriacigeorgica</i>	<u>2.04</u>	<u>2.07</u>	1.87	1.78	<i><1.70</i>	1.88	<i><1.70</i>	<i><1.70</i>
<i>N. farcinica</i>	n/a	n/a	<u>2.19</u>	<u>2.23</u>	<u>2.04</u>	<u>1.95</u>	<u>2.06</u>	<u>2.12</u>
<i>N. farcinica</i>	n/a	n/a	<u>2.03</u>	<u>2.47</u>	<u>2.06</u>	<u>2.16</u>	<u>2.18</u>	<u>2.03</u>
<i>N. farcinica</i>	n/a	n/a	<u>2.31</u>	<u>2.38</u>	<u>2.16</u>	<u>2.06</u>	<u>2.07</u>	<u>2.14</u>
<i>N. nova</i>	n/a	n/a	<u>2.26</u>	<u>2.19</u>	<u>2.40</u>	<u>2.36</u>	<u>2.31</u>	<u>2.35</u>
<i>N. nova</i>	n/a	n/a	<u>2.37</u>	<u>2.46</u>	<u>2.48</u>	<u>2.49</u>	1.89	<u>2.40</u>
<u>CBA at 35 °C</u>								
<i>N. abscessus</i>	n/a	n/a	<u>2.17</u>	<u>2.14</u>	<u>2.09</u>	1.89	1.70	1.80
<i>N. abscessus</i>	n/a	n/a	<u>1.84</u>	<u>2.03</u>	<u>1.89</u>	1.95	1.96	1.82
<i>N. cyriacigeorgica</i>	<u>2.37</u>	<u>2.42</u>	<u>2.36</u>	<u>2.31</u>	<u>2.08</u>	<u>2.22</u>	<i><1.70</i>	1.96
<i>N. cyriacigeorgica</i>	<u>2.00</u>	<u>2.03</u>	<u>1.95</u>	<u>1.87</u>	<i><1.70</i>	<i><1.70</i>	<i><1.70</i>	<i><1.70</i>
<i>N. cyriacigeorgica</i>	<u>2.05</u>	<u>2.12</u>	1.89	1.93	1.73	<i><1.70</i>	1.77	1.84
<i>N. farcinica</i>	n/a	n/a	<u>2.08</u>	<u>2.08</u>	<u>2.02</u>	1.85	1.98	1.93
<i>N. farcinica</i>	n/a	n/a	<u>2.21</u>	<u>2.23</u>	<u>2.04</u>	<u>2.01</u>	<u>2.13</u>	<u>2.08</u>
<i>N. farcinica</i>	n/a	n/a	<u>2.21</u>	<u>2.21</u>	<u>2.14</u>	<u>1.97</u>	<u>1.97</u>	<u>1.89</u>
<i>N. nova</i>	n/a	n/a	<u>2.47</u>	<u>2.38</u>	<u>2.35</u>	<u>2.22</u>	<u>2.35</u>	<u>2.37</u>
<i>N. nova</i>	n/a	n/a	<u>2.40</u>	<u>2.27</u>	<u>2.28</u>	<u>2.30</u>	<u>2.19</u>	<u>2.25</u>

Extractiemethode

Comparison of extraction methods and database versions for individual *Nocardia* species identification.

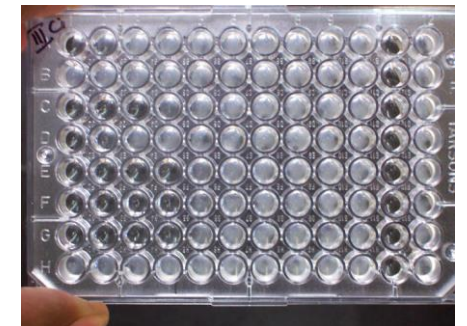
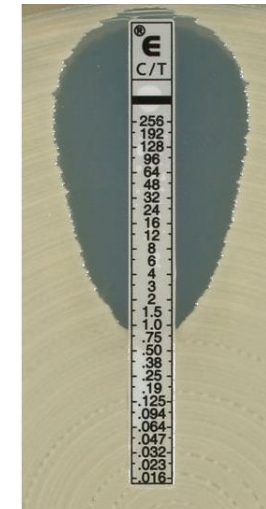
	Mycobacterial extraction (n)					Ethanol formic acid extraction (n)					Direct on-target extraction (n)				
	No. of isolates (n = 39)	BDAL v5		BDAL v6		No. of isolates (n = 60)	BDAL v5		BDAL v6		No. of isolates (n = 60)	BDAL v5		BDAL v6	
		≥1.8	≥2.0	≥1.8	≥2.0		≥1.8	≥2.0	≥1.8	≥2.0		≥1.8	≥2.0	≥1.8	≥2.0
<i>N. abscessus</i>	4	3	0	4	4	4	4	1	4	3	4	4	1	4	4
<i>N. asteroides complex</i> *	3	1	1	2	1	9	5	2	7	5	9	6	4	8	5
<i>N. beijingensis/asiatica</i> ^#	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0
<i>N. brasiliensis</i>	0	0	0	0	0	3	2	0	2	0	3	2	0	2	0
<i>N. cyriacigeorgica</i>	5	3	2	3	2	6	4	3	4	3	6	5	4	5	4
<i>N. farcinica</i>	7	6	2	6	3	11	11	7	11	7	11	11	9	11	9
<i>N. kruczakiae</i>	1	1	0	1	0	2	1	0	1	0	2	0	0	0	0
<i>N. nova</i>	9	6	2	8	4	14	12	10	13	12	14	13	6	14	8
<i>N. otitidiscavarium</i>	0	0	0	0	0	1	1	1	1	1	1	1	0	1	0
<i>N. paucivorans</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>N. puris</i> §	1	0	0	1	1	1	0	0	1	1	1	0	0	1	1
<i>N. rhamnosiphila</i> #	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0
<i>N. takedensis</i>	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0
<i>N. testacea</i>	1	1	0	1	0	1	1	0	1	0	1	0	0	0	0
<i>N. veterana</i>	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1
<i>N. vinacea</i> #	2	0	0	0	0	2	0	0	0	0	2	0	0	0	0
<i>N. wallacei</i> §	1	0	0	1	0	1	0	0	1	1	1	0	0	1	1
Total no. correctly identified (%)		22 (56)	8 (21)	28 (72)	16 (41)		43 (72)	26 (43)	48 (80)	35 (58)		44 (73)	26 (43)	49 (82)	34 (57)
Total no. incorrectly identified (%)		0 (0)	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)	0 (0)	0 (0)		0 (0)	0 (0)	0 (0)	0 (0)
Total no. below score threshold (%)		17 (44)	31 (79)	11 (28)	23 (59)		17 (28)	34 (57)	12 (20)	25 (42)		16 (27)	34 (57)	11 (18)	26 (43)

Besluit vraag 1

- MALDI-TOF MS is heden een volwaardig alternatief!
- MALDI-TOF MS op vroegtijdige koloniegroei:  % correcte identificatie
- geen meerwaarde van lange extractiemethode
- indien te lage score  gen-sequentieanalyse

Vraag 2: Welke methode moet gebruikt worden voor gevoeligheidsbepaling van Nocardia?

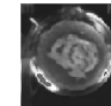
- **Isolatie Nocardia** uit klinisch respiratoir staal = **meestal reële infectie** en geen kolonisatie
- Uitvoeren **antibiogram zeer sterk aangeraden** gezien **variabel** inter-species **resistentiepatroon**
- Opzet: literatuurstudie: Etest vs BMD (4 artikels)
- Referentiemethode volgens CLSI: broth microdilution (BMD)
- CLSI criteria
 - categorische en essentiële overeenkomst moeten $\geq 90\%$ zijn
 - het aantal VME moet $\leq 3\%$ van het totaal aantal resistente stammen
 - het aantal ME moet $\leq 3\%$ van het totaal aantal geteste stammen



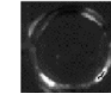
CLSI: BMD

Step	Action	Comment
1.	Using a loop or sterile applicator stick to sweep the area of confluent growth on the agar, prepare a heavy organism suspension in a small volume of sterile, deionized water or saline in a microcentrifuge tube or a small culture tube with glass beads.	For <i>Nocardia</i> spp. and aerobic actinomycetes other than <i>R. equi</i> , prepare the inoculum from growth on blood or trypticase soy agar after incubating for 3–5 days (or until growth is sufficient) at 35°C ± 2°C in ambient air.
2.	Break up organism clumps using a micropestle in a microcentrifuge tube and/or using glass beads. For the latter, prepare the suspension in a small culture tube using 7–10 3-mm glass beads, vortexing vigorously until clumps are broken up.	
3.	Allow the suspension to sit for 10–15 minutes to allow any remaining large clumps to settle to the bottom of the tube.	
4.	Using a Pasteur pipette, add several drops of the supernatant from the heavy suspension to a tube containing 2 mL water or saline.	Use tubes compatible with the nephelometer or spectrophotometer.
5.	Check the suspension's turbidity and adjust to make a suspension equivalent to that of a 0.5 McFarland standard.	If there is a range of readings for the nephelometer or spectrophotometer being used that correspond to a 0.5 McFarland suspension, the organism suspension's turbidity should be in the middle of the range to allow for variation in organism concentration related to colony characteristics. NOTE: The suspension approximating the 0.5 McFarland standard can also be made from a broth culture. Large organism clumps should be broken up by vortexing with glass beads, as described in step 2. Allow the broth suspension to sit for approximately 15 minutes to allow any remaining large clumps to settle to the bottom of the tube.¹¹⁹

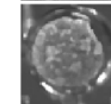
- inoculum = 0,5 McFarland
- 50 µL in 10 mL CAMHB -> 100 µL per well
- incubatie in omgevingslucht op 35 °C
- groei controle na 48-72u
- + TMP-SMX schijfje ter controle
 - ≥ 35 mm inhibitie = gevoelig
 - ≤ 15 mm inhibitie = resistent



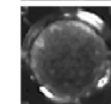
Positive Control



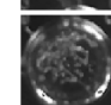
Negative Control



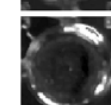
4+ Heavy growth, comparable to the positive control



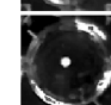
3+ Heavy growth or turbidity



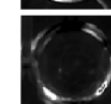
2+ Moderate countable colonies or moderate turbidity



1+ Haze or slight turbidity



+/- Few countable colonies or minimal turbidity



No growth

Biomerieux®: Etest

MYCOBACTERIA AND AEROBIC ACTINOMYCETES ¹⁾

ORGANISM	SPECIFIC PHENOTYPE	MEDIA ⁶⁾	INOCULUM		SUGGESTED MIC PANEL ³⁾	INCUBATION		RECOMMENDED QUALITY CONTROL		COMMENTS ⁴⁾
			McF equivalent	Suspension medium		Time (h)	Temperature/ Atmosphere	Strain	ATCC®	
<i>Mycobacterium tuberculosis</i>		Middlebrook 7H11 + 10% OADC agar	3-4	M7H9 broth + 0.5% tween + 2% glycerol Vortex with sterile glass beads 3-5 mins, settle 20 mins & adjust supernatant to correct turbidity.	Ethambutol (EB) Ethionamide (ET) Isoniazide (IZ) Rifampicin (RI)	5-10 days	37 ± 2°C 5-10% CO ₂	<i>Mycobacterium tuberculosis</i> <i>M. tuberculosis</i>	27294™ AW388 (not ATCC)	- Pre-incubation 24h - 1 strip/90mm plate, seal plates - Perform all work within BSC class IIA
Nontuberculous mycobacteria		MHA + 10% OADC + 5% blood (<i>M. kansasii</i> use same agar as for MTB)	1 (3 for <i>M. kansasii</i>)	M7H9 broth Vortex with sterile glass beads 3-5 mins, settle 20 mins & adjust supernatant to correct turbidity.	Amikacin (AK) Ciprofloxacin (CI) Clarithromycin (CH) Rifampicin (RI)	5-10 days	35 ± 2°C (<i>M. marinum</i> at 30°C) 5% CO ₂	<i>M. avium</i> <i>M. avium</i> spp. <i>avium</i> <i>M. marinum</i> <i>M. kansasii</i>	700898™ 35713™ 927™ 12478™	- 1 strip/90mm plate, seal plates - Antibiogram is species specific
Rapid growing mycobacteria		MHA + 5% blood	1	Saline	Cefoxitin (FX) Imipenem (IP) Ciprofloxacin (CI) Clarithromycin (CH)	48-72h	30-35°C Ambient air, moist	<i>M. fortuitum</i> <i>M. peregrinum</i>	6841™ 700686™	- Subculture twice before preparing inoculum - 3-4 strips max./150mm plate to facilitate reading
<i>Nocardia</i> spp.		MHA + 5% blood	1	Broth (BHI broth)	Amikacin (AK) Trimethoprim/sulfamethoxazole (TS) Ciprofloxacin (CI) Clarithromycin (CH) Imipenem (IP)	48-72h (dependent on spp.)	35 ± 2°C Ambient air	<i>S. aureus</i>	29213™	

Overzicht artikels

studie jaar publicatie	n (aantal stammen)	techniek	inoculum	incubatie			geteste antibiotica	breekpunten
				tijd	temperatuur	atmosfeer		
Biehle et al. 1994	52 + 3 ATCC	BMD: NCCLS 1992 M7-A2 Etest: volgens fabrikant (MHA)	BMD: 0,5 McF Etest: 0,5 McF	BMD: 24, 48 & 72u Etest: 72u	35°C	omgevingslucht	amikacine, amoxicilline-clavulaanzuur, cefotaxime, ceftriaxone, ciprofloxacin, minocycline, imipenem en TMP-SMX	NCCLS criteria voor aerobe bacteriën: M100-S4
Ambaye et al. 1997	23 + 3 ATCC	BMD: NCCLS 1993 M7-A3 Etest: volgens fabrikant (MHA)	BMD: 0,5-1 McF Etest: 0,5-1 McF	BMD: 72u Etest: 72u	35°C	omgevingslucht	amikacine, ampicilline, amoxicilline-clavulaanzuur, ceftriaxone, ciprofloxacin erythromycin, imipenem, minocycline en TMP-SMX	NCCLS criteria voor aerobe bacteriën: M100-S5
Lowman et al. 2010	39 + 4 ATCC	BMD: CLSI 2006 M7-A7 Etest: volgens fabrikant (MHA)	BMD: 0,5 McF Etest: 0,5 McF	BMD: 24, 48 & 72u Etest: 24, 48 & 72u	35°C	omgevingslucht	amikacine, amoxicilline-clavulaanzuur, ceftriaxone, ciprofloxacin, claritromycine, imipenem, TMP-SMX en linezolid	NCCLS criteria voor aerobe actinomycetes: M24-A1 (attachment 1)
Brown-Elliott et al. 2016	87 + 4 ATCC	BMD: CLSI 2011 M24-A2 Etest: volgens fabrikant (MHA)	BMD: 0,5 McF Etest: 0,5 McF	onbekend	35°C	omgevingslucht	ertapenem, imipenem, meropenem	CLSI criteria voor aerobe actinomycetes: M62-A1 (attachment 2)

Drug	No. of Strains Tested	Number of E-Test MICs ^a Within Indicated Log ₂ Concentration of Broth Microdilution MICs ^b											% Agreement Within	
		> -2	-2	-1.5	-1	-0.5	Same	+0.5	+1	+1.5	+2	> +2	+1 Log ₂ Concentration	±1.5 Log ₂ Concentration
Trimethoprim-sulfamethoxazole ^c	52	2	3	1	6	9	20	1	7	1	0	2	82.7	88.5
Amoxicillin-clavulanic acid ^d	52	0	1	2	10	15	13	2	7	0	2	0	90.4	94.2
Imipenem	52	0	0	2	14	8	17	4	5	1	1	0	92.3	98.1
Ciprofloxacin	52	0	2	4	8	5	9	6	15	0	2	1	82.7	90.4
Minocycline	52	0	2	0	8	10	14	7	8	2	1	0	90.4	94.2
Cefotaxime	52	0	0	2	7	6	30	0	5	0	1	1	92.3	96.2
Ceftriaxone	52	0	1	0	7	11	24	4	3	1	0	1	94.2	96.2
Amikacin	52	0	0	3	3	9	18	10	7	1	1	0	90.4	98.1
Overall agreement within 1 log ₂ dilution														
Overall agreement within 1.5 log ₂ dilutions														

372/416 = 89.4%

392/416 = 94.2%

^aE Test MICs determined by use of Mueller-Hinton agar.

^bBroth microdilution MICs determined by use of Mueller-Hinton broth.

^cTrimethoprim-sulfamethoxazole ratio, 1:19.

^dAmoxicillin-clavulanic acid ratio, 2:1.

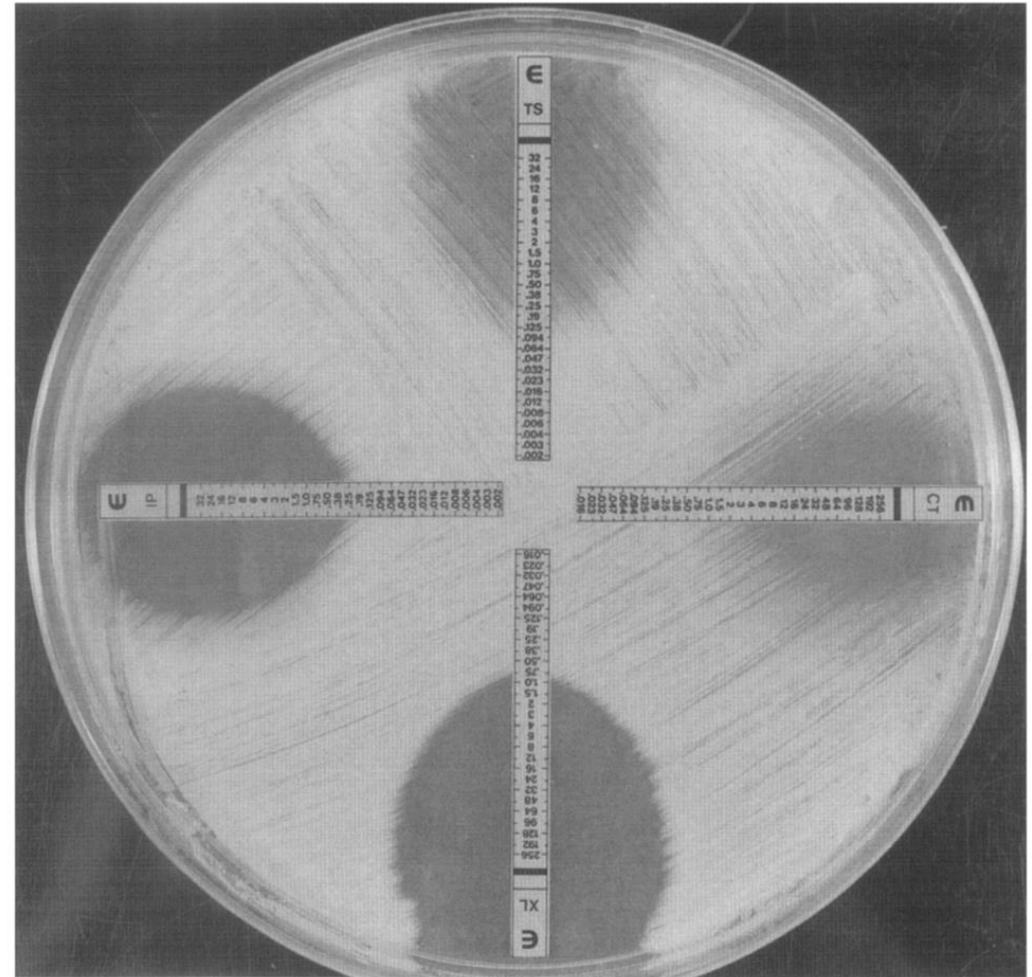
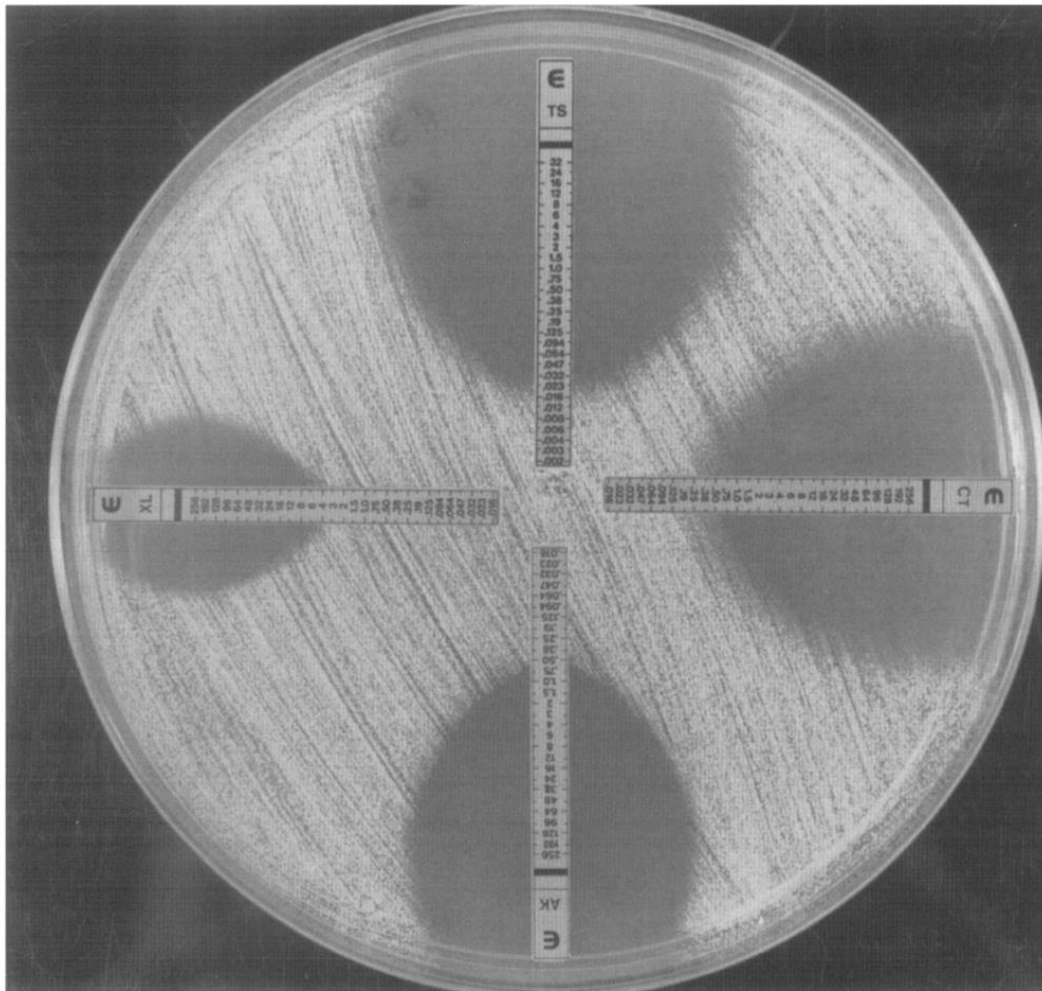
Antimicrobial Agent	E Test vs Disk Diffusion			E Test vs Broth Microdilution		
	S ^a	I	R	S	I	R
Trimethoprim-sulfamethoxazole	100 (48/48) ^b	—	100 (4/4)	100 (48/48)	—	100 (4/4)
Amoxicillin-clavulanic acid	100 (29/29)	33 (4/12)	55 (6/11)	97 (36/37)	67 (6/9)	100 (6/6)
Imipenem	100 (39/39)	0 (0/1)	100 (12/12)	100 (39/39)	—	92 (12/13)
Ciprofloxacin	100 (25/25)	40 (2/5)	86 (19/22)	92 (22/24)	50 (3/6)	100 (22/22)
Minocycline	98 (48/49)	0 (0/3)	—	96 (49/51)	100 (1/1)	—
Cefotaxime	100 (36/36)	—	100 (16/16)	100 (36/36)	0 (0/1)	100 (15/15)
Ceftriaxone	100 (35/35)	33 (1/3)	86 (12/14)	97 (35/36)	50 (1/2)	93 (13/14)
Amikacin	100 (45/45)	—	100 (7/7)	100 (45/45)	—	100 (7/7)
% Agreement for all drugs	99 (305/306)	29 (7/24)	88 (76/86)	98 (310/316)	58 (11/19)	98 (79/81)
Overall agreement		93.2% (388/416)			96.2% (400/416)	

^aS, susceptible; I, intermediate; and R, resistant.

^bNumbers in category/total tested.

^cNCCLS criteria for rapidly growing bacteria used to define susceptibility.

Antimicrobial Agent	E Test vs Microbroth Dilution			
	% Complete Agreement	% Errors		
		Very Major	Major	Minor
Trimethoprim-sulfamethoxazole	100	0	0	0
Amoxicillin-clavulanic acid	92	0	0	7.6
Imipenem	98	0	0	1.9
Ciprofloxacin	90	0	0	9.6
Minocycline	96	NA ^c	0	3.8
Cefotaxime	98	0	0	1.9
Ceftriaxone	94	0	0	5.8
Amikacin	100	0	0	0
Total	96.2	0	0	3.8



Antimicrobial agent	Broth microdilution method (BMD)				BMD vs Etest					
	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)	Range (µg/ml)	% Susceptible ^b	% Categorical agreement	% Essential agreement	Correlation coefficient	% of errors		
								Very major	Major	Minor
Amikacin ^c	0.25	0.5	<0.12–2	100.0	100.0	65.9	0.79	0.0	0.0	0.0
Amoxicillin-clavulanate ^d	16	>64	1–>64	45.0	79.5	46.2	0.81	11.8	5.9	12.8
Ceftriaxone ^c	4	256	<0.5–>256	61.9	67.5	60.0	0.64	0.0	12.0	25.0
Ciprofloxacin ^c	4	64	0.25–>64	20.9	90.2	46.3	0.72	3.3	0.0	7.3
Clarithromycin ^c	32	>64	<0.12–>64	30.2	73.2	61.0	0.25	3.7	33.3	14.6
Imipenem ^c	8	>64	<0.12–>64	48.8	82.9	61.0	0.97	5.6	0.0	14.6
Trimethoprim- sulfamethoxazole ^c	0.12	0.5	<0.015–1	100.0	100.0	53.7	0.66	0.0	0.0	0.0
Linezolid ^c	2	2	0.25–4	100.0	100.0	81.6	0.51	0.0	0.0	0.0

^a Includes reference strains.

^b Susceptibility based on NCCLS interpretive criteria (17).

^c 43 MICs evaluated; ATCC 19247 tested twice on separate occasions. No influence on MIC₅₀, MIC₉₀, or range.

^d 40 MICs evaluated.

^e 42 MICs evaluated.

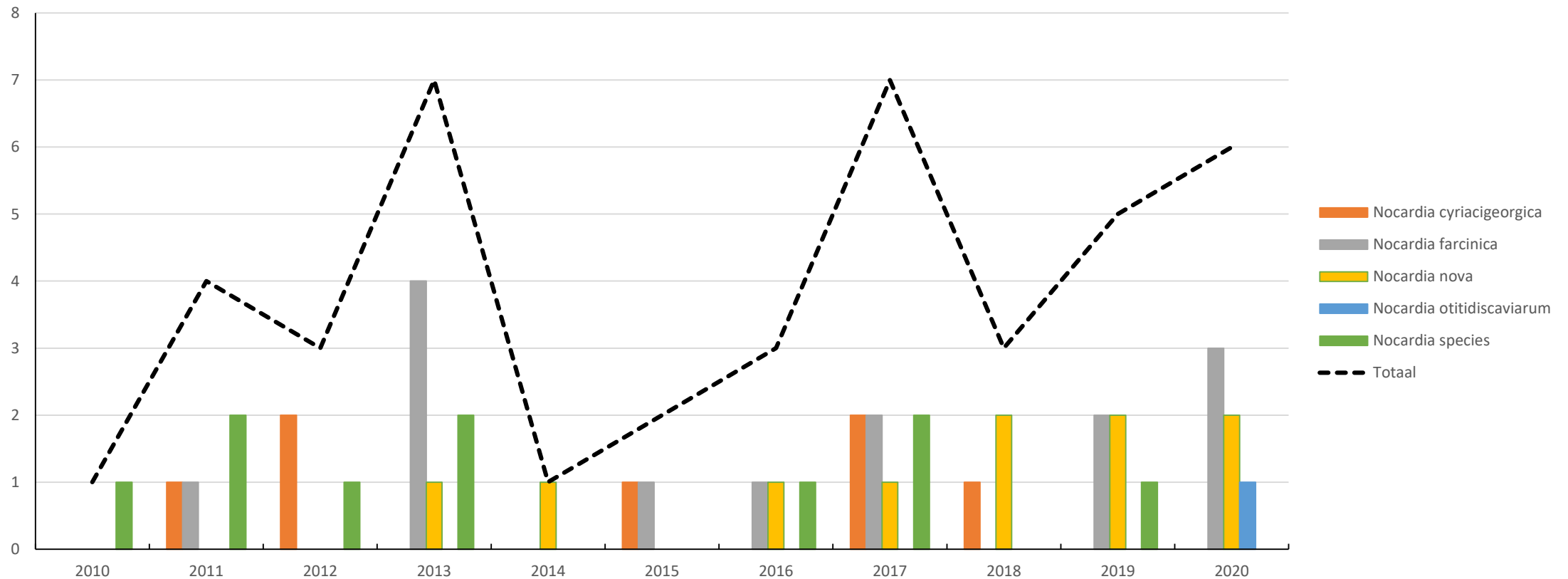
Species or complex and drug	No. of isolates tested	% error		
		Very major	Major	Minor
<i>M. fortuitum</i>				
Ertapenem	22	5	0	0
Meropenem	22	0	0	86
Imipenem	22	0	5	36
<i>M. abscessus</i> subsp. <i>abscessus</i>				
Ertapenem	44	0	0	0
Meropenem	44	0	0	14
Imipenem	44	0	7	48
<i>M. chelonae</i>				
Ertapenem	13	0	0	0
Meropenem	12	0	0	0
Imipenem	12	0	0	75
<i>N. cyriacigeorgica</i>				
Ertapenem	10	0	0	10
Meropenem	10	0	0	20
Imipenem	11	0	36	18
<i>N. nova</i> complex				
Ertapenem	32	0	3	43
Meropenem	31	0	0	9
Imipenem	32	0	0	0
<i>N. brasiliensis</i>				
Ertapenem	13	0	0	31
Meropenem	13	0	8	31
Imipenem	13	0	0	8
<i>N. transvalensis</i> complex				
Ertapenem	12	33	8	17
Meropenem	12	8	8	42
Imipenem	12	8	0	25

Besluit vraag 2

- BMD = gouden standaard
- BMD versus Etest: moeilijke vergelijking
 - beperkte literatuur
 - $\neq$ inoculum & bodem t.o.v. huidige richtlijnen
- nood aan eigen vergelijkende studie
- quid karakterisering van stammen bij retrospectieve studies o.b.v. Etest?

Vraag 3. Welke methode is het meest geschikt om gevoeligheidsbepaling in UZ Leuven uit te voeren?

Aantal patiënten met nocardiose 2010-2020 (n= 42)



Gevoeligheidspatroon o.b.v. species identificatie

	N. abscessus	N. brasiliensis	N. cyriacigeorgica	N. farcinica	N. nova complex	N. otitidiscaviarum	N. transvalensis complex
Amikacine (CLSI)	S	S	S	S	S	S	R
Amoxicilline	S	R	R	R	S	R	V
Amoxcilline-clavulaanzuur (CLSI)	S	S	R	S	R	R	V (meestal R)
Ceftriaxone (CLSI)	S	V (meestal R)	S	R	S	R	S
Ciprofloxacin (CLSI)	R	R	R	S	R	S	S
Clarithromycine (CLSI)	R	R	R	R	S	V	R
Imipenem (CLSI)	V	R	S	V (meestal S)	S	R	V (meestal R)
Linezolid (CLSI)	S	S	S	S	S	S	S
Minocycline (CLSI)	V (meestal S)	S	V (meestal R)	V (meestal R)	V (meestal R)	V (meestal R)	V (meestal R)
Moxifloxacin	V (meestal R)	S	R	S	R	V (meestal R)	S
Tigecycline	S	S	V	R	V	X	X
Tobramycine (CLSI)	V (meestal S)	S	S	R	R	V	R
TMP-SMX (CLSI)	S	S	S	S	S	S	S

Vergelijking commerciële platen

Nocardia plaat

	1	2	3	4	5	6	7	8	9	10	11	12
A	CLA 0.03	CLA 0.06	CLA 0.12	CLA 0.25	CLA 0.5	CLA 1	CLA 2	CLA 4	CLA 8	CLA 16	AUG2 2/1	AUG2 4/2
B	AUG2 8/4	AUG2 16/8	AUG2 32/16	AUG2 64/32	AUG2 128/64	AMI 0.25	AMI 0.5	AMI 1	AMI 2	AMI 4	AMI 8	AMI 16
C	AMI 32	IMI 0.12	IMI 0.25	IMI 0.5	IMI 1	IMI 2	IMI 4	IMI 8	IMI 16	IMI 32	AXO 1	AXO 2
D	AXO 4	AXO 8	AXO 16	AXO 32	AXO 64	AXO 128	LZD 0.5	LZD 1	LZD 2	LZD 4	LZD 8	LZD 16
E	MXF 0.015	MXF 0.03	MXF 0.06	MXF 0.12	MXF 0.25	MXF 0.5	MXF 1	MXF 2	MXF 4	MXF 8	MIN 0.06	MIN 0.12
F	MIN 0.25	MIN 0.5	MIN 1	MIN 2	MIN 4	MIN 8	MIN 16	DOX 0.12	DOX 0.25	DOX 0.5	DOX 1	DOX 2
G	DOX 4	DOX 8	DOX 16	TOB 2	TOB 4	TOB 8	TOB 16	TOB 32	SXT 0.06/1.19	SXT 0.12/2.38	SXT 0.25/4.75	SXT 0.5/9.5
H	SXT 1/19	SXT 2/38	SXT 4/76	SXT 8/152	CIP 0.12	CIP 0.25	CIP 0.5	CIP 1	CIP 2	CIP 4	CIP 8	POS

RAPMYCOI plaat

	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



Imipenem vs. meropenem

Complex or species and drug	Intermediate breakpoint (µg/ml)	No. of isolates tested	MIC (µg/ml)			% susceptible/intermediate
			Range	50%	90%	
<i>N. cyriacigeorgica</i>						
Imipenem	8	25	≤1–32	8	>16	60
Meropenem	8	25	4–>16	8	>16	68
Ertapenem	4 ^a	26	2–>16	8	>16	15
<i>N. nova</i> complex						
Imipenem	8	57	≤0.5–8	≤1	2	100
Meropenem	8	54	≤0.5–16	≤1	4	94
Ertapenem	4 ^a	57	0.5–16	2	4	96
<i>N. abscessus</i>						
Imipenem	8	13	2–32	>16	32	23
Meropenem	8	11	1–8	2	4	100
Ertapenem	4 ^a	13	0.5–4	2	4	100
<i>N. brasiliensis</i>						
Imipenem	8	23	16–>32	>16	>32	0
Meropenem	8	23	4–>16	>16	>16	48
Ertapenem	4 ^a	23	4–>16	>16	>16	26
<i>N. farcinica</i>						
Imipenem	8	19	≤1–>16	8	>16	63
Meropenem	8	18	4–>16	8	>16	33
Ertapenem	4 ^a	19	4–>16	8	16	21
<i>N. transvalensis</i> complex						
Imipenem	8	18	4–>32	16	>32	22
Meropenem	8	18	2–16	8	16	83
Ertapenem	4 ^a	18	2–>16	>16	>16	22

Reproduceerbaarheid BMD

Antimicrobial susceptibility testing (AST) of clinical isolates of *Nocardia* is recommended to detect resistance to commonly used antimicrobial agents; such testing is complicated by difficulties in inoculum preparation and test interpretation. In this study, six laboratories performed repetitive broth microdilution testing on single strains of *Nocardia brasiliensis*, *Nocardia cyriacigeorgica*, *Nocardia farcinica*, *Nocardia nova*, and *Nocardia wallacei*. For each isolate, a total of 30 microdilution panels from three different lots were tested at most sites. The goal of the study was to determine the inter- and intralaboratory reproducibility of susceptibility testing of this group of isolates. Acceptable agreement (>90% agreement at ± 1 dilution of the MIC mode) was found for amikacin, ciprofloxacin, clarithromycin, and moxifloxacin. After eliminating MIC values from single laboratories whose results showed the greatest deviation from those of the remaining laboratories, acceptable agreement was also found for amoxicillin-clavulanic acid, linezolid, minocycline, and tobramycin. Results showed unsatisfactory reproducibility of broth microdilution testing of ceftriaxone with *N. cyriacigeorgica* and *N. wallacei*, tigecycline with *N. brasiliensis* and *N. cyriacigeorgica*, and sulfonamides with *N. farcinica* and *N. wallacei*. *N. nova* ATCC BAA-2227 is proposed as a quality control organism for AST of *Nocardia* sp., and the use of a disk diffusion test for sulfisoxazole is proposed as a check of the adequacy of the inoculum and to confirm sulfonamide MIC results.

TABLE 8 Intralaboratory reproducibility

Test site	Total no. of susceptibility results	No (%) of readings outside mode ± 1 dilution	No (%) of readings spanning >3 dilutions ^a
1	1,901	125 (6.6)	7 (10.8)
2	1,924	131 (6.8)	4 (6.2)
3	1,776	54 (3.0) ^b	2 (3.1)
4	1,893	149 (7.8)	8 (12.3)
5	1,934	277 (14.4) ^c	11 (16.9)
6	1,950	237 (12.2) ^c	24 (36.9) ^d

^a The total number of antimicrobial-organism combinations is 65.

^b $P < 0.001$ (see text).

^c $P < 0.001$ (see text).

^d $P < 0.01$ compared to sites 1 through 4 and $P = 0.035$ compared to site 5.

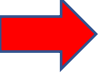
Financiële balans

	Kostprijs in € (incl. btw)
Etest® (o.b.v.Nocardia plaat)	55
Etest® (o.b.v. RAPMYCOI plaat)	64
commerciële plaat	136

550734 550745 Bepaling van de gevoeligheid voor antibacteriële stoffen van
aerobe kiemen, andere dan mycobacteriën, na identificatie B 400
(Maximum 1)

550874 550885 Bepaling van de minimale inhiberende concentratie van
antibacteriële stoffen bij kiemen geïsoleerd uit bloed of
cerebrospinaal vocht, per antibioticum B 400
(Maximum 3) (Diagnoseregul 52)

Besluit vraag 3

- belang terugkoppeling empirische R/ o.b.v. species identificatie
- laag # stalen  nood aan centraal uitvoerend laboratorium (opbouw expertise!)
- geen specifieke terugbetaling voor Nocardia

