AMST hetkeseis ja tulevik kliinilises töös

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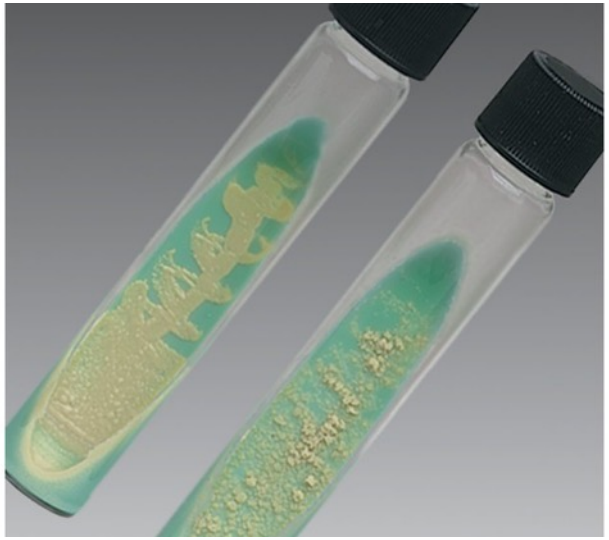
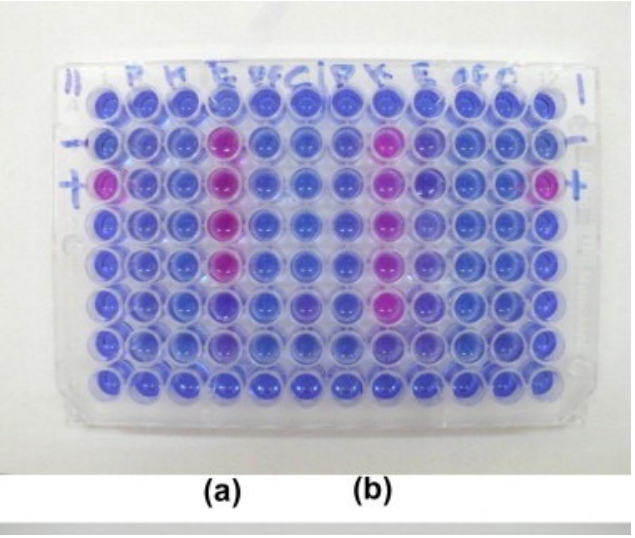
27.04.2021

Mükobakterite ravimtundlikkus

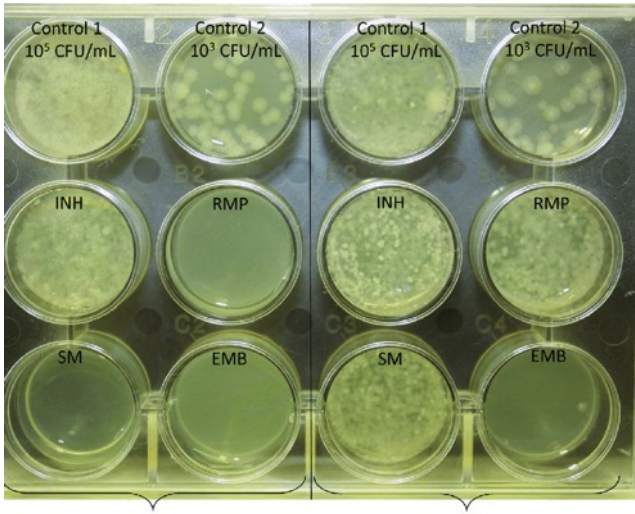
- Ajalooliselt
  - Põhineb proportsiooni meetodil
  - Enamus maailmast järgib WHO soovitusi
  - Kriitilised kontsentratsioonid on määratud valideerimisuuringutes
    - Sõltuvad suures osas bakterist ja ei arvesta PK/PD andmete ja võimalike ravimidoosidega
    - WHO on teinud kokkuvõtte oma soovitusteks
  - Igal söötmel oma kriitilised kontsentratsioonid

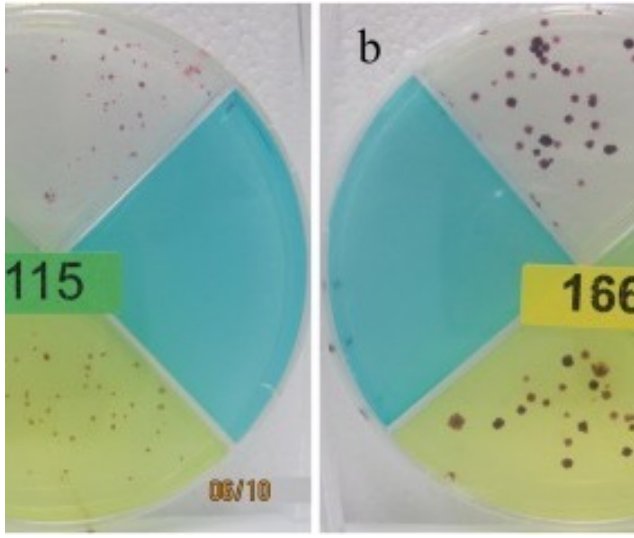




(a) (b)

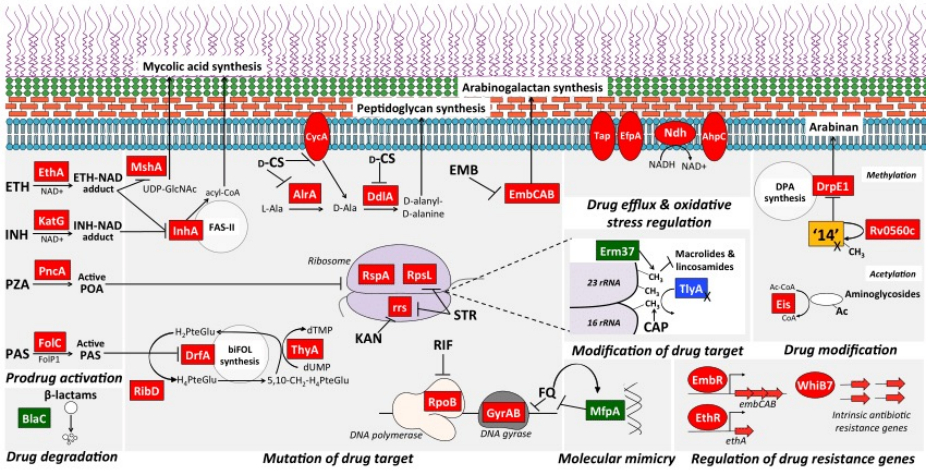

BACTEC MGIT 960
Unloaded AST Set Report
Temperature
A
B
END OF

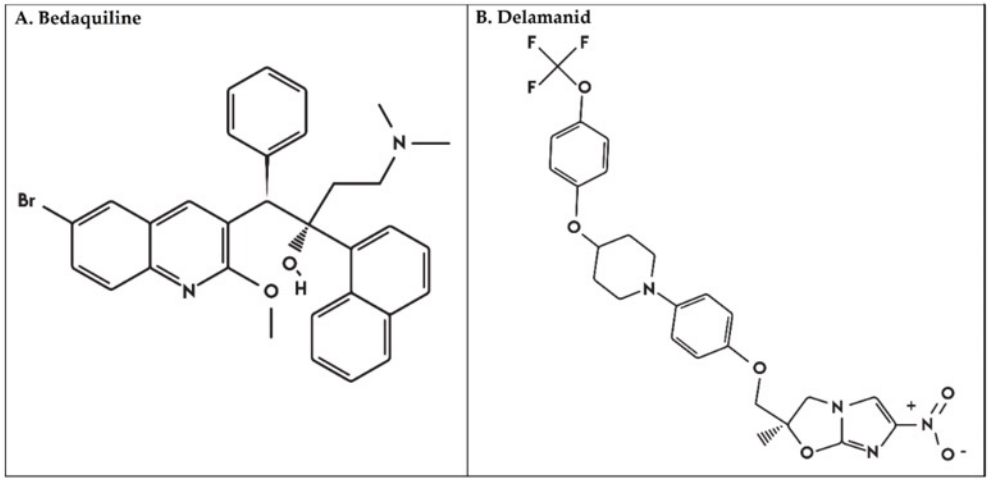

Control 1
10⁵ CFU/mL
Control 2
10³ CFU/mL
Control 1
10⁵ CFU/mL
Control 2
10³ CFU/mL
INH
RMP
INH
RMP
SM
EMB
SM
EMB
VNM7
VNM33


115
b
166

Miks muutused?

- Uute tuberkuloosiravimite kliinilised uuringud jõudsid lõpufaasi ja selgus, et ...
  - Ravimifirmad andsid EMAle hindamiseks väga segased ravimtundlikkuse andmed
  - EMA palus EUCASTil leida referentsmeetod
- WGS hüppeline areng
- Järjest enam iseloomustati ravimresistentsusega seotuid mutatsioone
- Järjest enam iseloomustatakse mutatsioone, mis ei põhjusta resistentsust, kuid on detekteeritavad molekulaarsete kiirtestide poolt
- Järjest enam leiti Geno/Fenotüübi erinevusi mida ei suudeta mõista





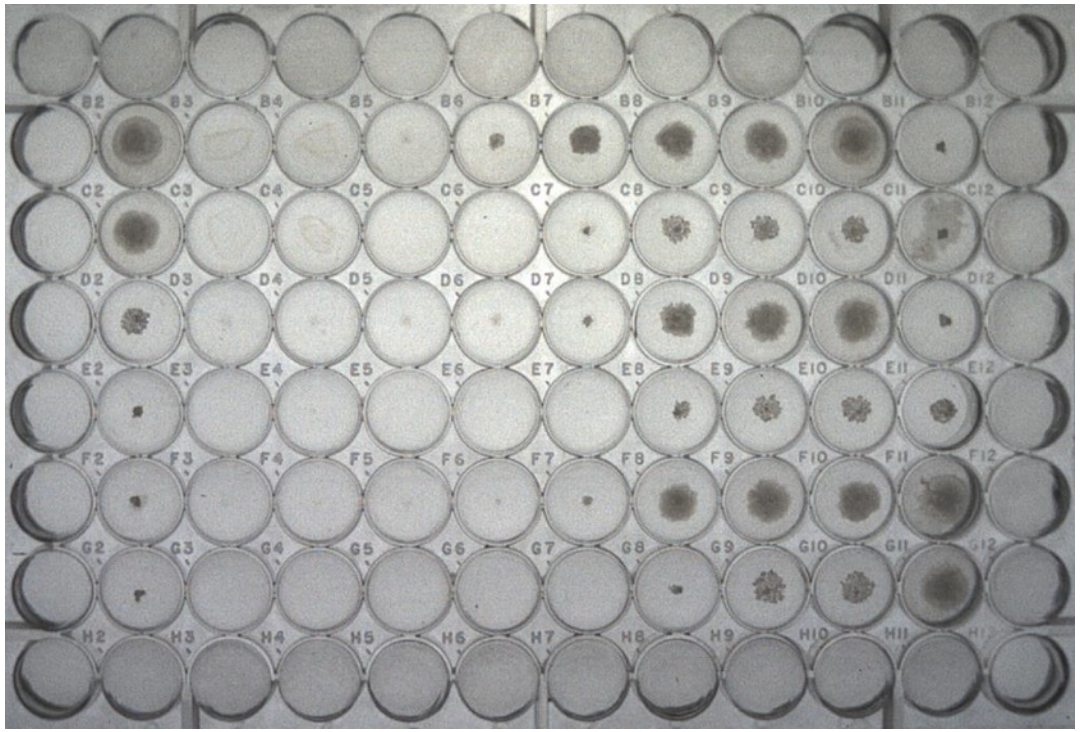
Group	Medicine	Abbreviation	Critical concentrations (µg/ml) for DST by medium			
			Löwen-stein Jensen[1]	Middle-brook 7H10[1]	Middle-brook 7H11[1]	BACTEC MGIT liquid culture[1]
Group A	Levofloxacin (CC)	LFX[2,3]	**2.0**	1.0	-	1.0
	Moxifloxacin (CC)	MFX[2,3]	**1.0**	0.5	0.5	0.25
	Moxifloxacin (CB)[4]			2.0	-	1.0
	Bedaquiline[5]	BDQ	-	-	**0.25**	**1.0**
	Linezolid[6]	LZD	-	1.0	1.0	1.0
Group B	Clofazimine	CFZ	-	-	-	**1.0**
	Cycloserine TerizidoneTerizidone	CS TZD	- -	- -	- -	- -
Group C	Ethambutol[7]	E	2.0	5.0	*7.5*	5.0
	Delamanid[8]	DLM	-	-	**0.016**	**0.06**
	Pyrazinamide[9]	PZA	-	-	-	100.0
	Imipenem-cilastatin Meropenem	IMP/CLN MPM	- -	- -	- -	- -
	Amikacin[10] (Or Streptomycin)	AMK (S)	30.0 4.0	2.0 2.0	- 2.0	1.0 1.0
	Ethionamide Prothionamide	ETO PTO	40.0 40.0	5.0 -	10.0 -	5.0 2.5
	Para-aminosalicylic acid	PAS	-	-	-	-

EUCAST reference 7H9 BMD

- Aitab 
  - Leida ECOFF-id kasutusel olevatele ravimitele
  - Leida MIC vahemikud olemasolevatele ravimitele
  - Mõista mutatsioonide ja MIC väärtuste seoseid
  - Toob selgust ja stabiilsust
  - Muudab uute ravimite testimise selgemaks
  - Paneb kommertstestide tootjad millestki sõltuma
  - Säästa raha
  - Utsitab WHOd kiiremini soovitustes muudatusi tegema (RIF BP muudatus)
- Ei aita 
  - Kiirendada tuberkuloosi diagnoosi, alustatakse tahkelt söötmelt
  - Meetod ei ole mõeldud rutiinkasutusse
  - Kalibreerimisprotokoll ei ole mõeldud rutiinlaborite jaoks, et nad iseseisvalt oma meetodeid kalibreerima hakkaksid
  - BMD meetodi lugemine peegliga nõuab kogenud/õppinud/kalibreeritud silmi

EUCAST reference 7H9 BMD

	1	2	3	4	5	6	7	8	9	10	11	12
A	200ul dH20	200ul dH20	200ul dH20	200ul dH20	200ul dH20	200ul dH20	200ul dH20	200ul dH20	200ul dH20	200ul dH20	200ul dH20	200ul dH20
B	negative control	GC 100%	AA1 (10-2) C8	AA1 (10-2) C7	AA1 (10-2) C6	AA1 (10-2) C5	AA1 (10-2) C4	AA1 (10-2) C3	AA1 (10-2) C2	AA1 (10-2) C1	GC 1%	200ul dH20
C	negative control	GC 100%	AA2 (10-2) C8	AA2 (10-2) C7	AA2 (10-2) C6	AA2 (10-2) C5	AA2 (10-2) C4	AA2 (10-2) C3	AA2 (10-2) C2	AA2 (10-2) C1	GC 1%	200ul dH20
D	negative control	GC 100%	AA3 (10-2) C8	AA3 (10-2) C7	AA3 (10-2) C6	AA3 (10-2) C5	AA3 (10-2) C4	AA3 (10-2) C3	AA3 (10-2) C2	AA3 (10-2) C1	GC 1%	200ul dH20
E	negative control	GC 1%	AA4 (10-2) C8	AA4 (10-2) C7	AA4 (10-2) C6	AA4 (10-2) C5	AA4 (10-2) C4	AA4 (10-2) C3	AA4 (10-2) C2	AA4 (10-2) C1	GC 100%	200ul dH20
F	negative control	GC 1%	AA5 (10-2) C8	AA5 (10-2) C7	AA5 (10-2) C6	AA5 (10-2) C5	AA5 (10-2) C4	AA5 (10-2) C3	AA5 (10-2) C2	AA5 (10-2) C1	GC 100%	200ul dH20
G	negative control	GC 1%	AA6 (10-2) C8	AA6 (10-2) C7	AA6 (10-2) C6	AA6 (10-2) C5	AA6 (10-2) C4	AA6 (10-2) C3	AA6 (10-2) C2	AA6 (10-2) C1	GC 100%	200ul dH20
H	200ul dH20	200ul dH20	200ul dH20	200ul dH20	200ul dH20	200ul dH20	200ul dH20	200ul dH20	200ul dH20	200ul dH20	200ul dH20	200ul dH20



Mida me senikaua teeme?

Mis saab, kui tuleb uus ravim?

Kes kalibreerib?

Kes MGIT MIC määrab?

Mis saab kui kasutad mingit muud ravimit?

Mida kalibreerida?

Kes maksab?

Kui kaua kalibreerimine aega võtab?

Kas sekveneerimine oleks tõhusam?

Kuna kalibreerib?

Kas referentsmeetodi MIC vahemikud on tüvest sõltuvad?

Mis võimalused meil on?

- Hakkame ikka ise EUCAST referentsmeetodit tegema?
- Vaatame ThermoFisheri poole?
- Lähme esimese rea ravimite puhul üle sekveneerimisele?
- Jätkame WHO soovituste järgi?
- MGIT kalibreerimine on liiga kulukas

Medicine	Initial diagnostic test	Phenotypic DST	Proposed Reference Method	Comment
Rifampicin	Xpert MTB/RIF Ultra	MGIT may not be reliable for certain isolates	DNA sequencing of the entire *rpoB* gene	Any mutation (excluding silent mutations) observed in the 81bp RRDR[a] hotspot region of the *rpoB* gene are known or assumed to be associated with rifampicin resistance. In a few cases, mutations in the *rpoB* gene outside the RRDR region are associated with rifampicin resistance. Patients require MDR-TB treatment.
Isoniazid	FL-LPA is the only WHO recommended rapid test for the detection of mutations in the *inhA* and *katG* genes. FL-LPA has a sensitivity of 85% for isoniazid resistance detection relative to MGIT DST. Specificity is high. Ideally, perform for all bacteriologically confirmed TB cases.	Reliable and reproducible when testing the CC in all media.	MGIT	If specific *inhA* promoter mutations are detected (and in the absence of any *katG* mutations), increasing the dose of isoniazid is likely to be effective; thus, additional isoniazid to a maximum dose of up to 15mg/kg per day can be considered Xpert MTB/RIF and line probe assays (LPA) are preferred to guide patient selection for the (H)RZE-Lfx regimen. Rifampicin resistance should be excluded before staring the HR-TB regimen and FQ resistance should be excluded as soon as possible
Ethambutol	No WHO recommended rapid method currently exists.	Phenotypic DST is not reliable and reproducible and is not recommended.	N/A	Genotypic DST (sequencing) maybe more reliable, than phenotypic DST. More evidence is needed
Pyrazi-namide	No WHO recommended rapid method currently exists.	DST method standardised in the MGIT. False resistant results can occur if DST inoculum not properly prepared	DNA sequencing of the *pncA* gene[b]	In a quality assured laboratory, a susceptible DST result for PZA can be used to guide the inclusion of PZA in a DR-TB treatment regimen. If PZA resistance is detected, do not include PZA if resistance is detected or if used do not count as an effective medicine

Lootused/ootused

- Thermo Fisher või mõni muu ettevõte toodab CE-IVD märgisega mikrotiiterplaadi TB-le õigete ravimite ja piisavate kontsentratsioonivahemikega
- Seda on lihtne kalibreerida ja sarnaneb enim referentsmeetodile
- Kui ravimid on plaadil lüofiliseeritud ei ole muret ka lahuste valmistamisega
- Murekohad
  - Tulemuste lugemine nõuab ikka kogenud silmi
  - Kas on võimalik ka vedelsöötmest alustada?
  - Plaadil ei saa olema Eestis kasutatavaid ravimeid mida WHO enam ei soovita

Aitäh!