Deeplex-MycTB, an all-in-one test for diagnosis and surveillance of drug-resistant TB

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MDR- and XDR- tuberculosis in 2014

- Estimated 480,000 new MDR-TB cases, incl. 46,000 XDR-TB cases; ~190,000 deaths
- Less than 30% diagnosed; less than 50% of those diagnosed receive appropriate treatment

"... serious **detection** and treatment gaps remain. Intensified efforts to close these gaps are urgently needed."
Whole-genome sequencing for prediction of Mycobacterium tuberculosis drug susceptibility and resistance: a retrospective cohort study

Training set of 2099 genomes correlated with phenotypes:
- 120 mutations as resistance determinant and 772 as benign in 14/23 candidate genes

Validation set of 1552 genomes using this catalog:
- 89.2% predicted phenotypes, 92.3% sensitivity and 98.4% specificity
- Superior sensitivity compared to 3 line probe assays

WGS promising for prediction of drug susceptibility and resistance
- Determinants concentrated in 14 candidate genes
Multicentric study on WGS from newly positive culture:

- Prediction of species and drug susceptibility with 93% accuracy
- Full WGS Dx, incl. genetic relatedness, median 21 days faster than classical Dx
- 7% less costly annually than current workflow

Primary culture still needed: delayed Dx

WGS on clinical samples: low multiplexing/coverage depth or capture system (Brown, JCM, 2015) not cost-effective (yet)
**Deeplex®-MycTB, an all-in-one NGS-based diagnostic test for *M. tuberculosis***

- Targeted NGS of single 24-plex amplification of main resistance targets, plus species identification and MTBC genotyping targets
- Deep sequencing for sensitive detection of heteroresistance
- Scalable up to 50 samples per MiSeq run, 70 samples per Miniseq run
- Fast, easy-to-use NGS data analysis and reporting on highly secured, high performance cloud

First-line drugs
- Pyrazinamide
- Isoniazid
- Ethambutol
- Rifampicin

Species identification
- Mycobacterial species

MTBC genotyping
- Spoligotyping
  + Phylogenetic SNPs

Second-line drugs
- Kanamycin
- Amikacin
- Streptomycin
- Capreomycin
- Fluoroquinolones
- Ethionamide

New 2nd line drugs for MTBC
- Bedaquiline, Clofazimine
- Linezolid

Total targets >13,000bp
- pncA
- ahpC, fabG1, katG, inhA
- embB
- rpoB
- hsp65
- ethA, inhA, fabG1
- eis
- rrs
- gidB, rpsL
- tlyA
- gyrA, gyrB
- rv0678
- rrl, rplC
- + Phylogenetic SNPs

Underlined: full genes

MTBC genotyping
- CRISPR/DR region
  + Phylogenetic SNPs

Species identification
- Mycobacterial species
Deeplex®-MycTB analysis

Cloud-based analysis

Cloud-based analysis

Visualisation, reporting

.pdf

Calibrated pipeline

Low frequency variant detection, depending on target position and coverage depth

Mycobacterial & non-mycobacterial species

Metagenomics

Mycobacterium *hsp65* DB (Dai et al., 2011)

Spoligotyping DB + phylogenetic SNPs

Resistance-associated mutation DBs

- PhyResSE, Feuerriegel et al. 2014
- Walker et al., 2015
- Miotto et al., 2015
- ReSeqTB, CRyPTIC (when accessible)

Mycobacterial & non-mycobacterial species

- *M. tuberculosis*
- MTBC
- *R. mucilaginosa*
- Gordoniaceae

SITVITWEB, Demay, 2012

PhyResSE, Feuerriegel, 2014
Deeplex®-MycTB: Target coverage depth and detection limit

Microscopy detection

On-target average coverage depths of 100x-1000x, down to 100-10 genomes
Deeplex®-MycTB: smear neg/culture pos MTBC sample

hsp65 sequence: full MTB match

MTB strain spoligotype: ST53-T1, Euro-American

Drug resistance targets

Metagenomics: MTBC

% match vs MTB type sequence

hsp65 sequence position

MTBC

Int. Ctrl

Mycobacterium

Coverage depth

Coverage depth
Deeplex®-MycTB: variant detection level

- Calibration by e.g. analysis of 220 drug susceptible and MDR isolates, plus internal controls
- Background nucleotide variation, incl. amplification and sequencing errors plus background biological variation below 2% except in few positions
- Likely sufficient in many cases as 1% phenotypically resistant might be an underestimate due to frequent counter-selection of resistant in culture due to fitness cost

![Graph with markers for new predicted determinants, known determinants, probable mixed strains, and in South African MDR strains not in Xpert. The graph shows nucleotide position on the x-axis and % 1st variant on the y-axis. A zoom-in feature is also indicated.]
Deeplex®-MycTB: semi-quantitative detection of variants

Mixtures of Asp94Gly:wt in gyrA (coll. ITG, Antwerp) in triplicated Deeplex tests

Semi-quantitative detection down to close to 1%
Deeplex®-MycTB evaluation and beta-testing

- In-house evaluation on clinical specimens:
  - Effective on most smear-pos and on ≈25% smear-neg, culture-pos specimens
- Evaluation of sensitivity and specificity on WHO-TDR MTBC strain bank:
  - 229 MTBC samples of diverse phenotypic and genetic resistance profiles and geographic origins
  - Previously used to evaluate the CEPHEID GeneXpert MTB/RIF
- 550 clinical samples from WHO drug resistance surveys in Djibouti and DRC (coll. WHO Supranational Reference Labs D. Cirillo, Milano, and B. De Jong, Antwerp)
Deeplex®-MycTB evaluation and beta-testing

- Preliminary Deeplex results on DNA extracts from clinical samples from Djibouti:
  - Analysis done on equivalent of 100 µl of clinical sample (vs 500-1,000 µl by Xpert)
  - 270/300 samples with sufficient on-target coverage on first run (7-9/49 invalid by Hain tests)
  - 15 predicted MDR-TB
  - Among 263 Rif –S by Xpert, only one discordance (with RpoB Ser531Leu, Ile561Val) among samples with Deeplex results
  - Among 36 Rif-R by Xpert, 27 confirmed and 7 Rif-S by Deeplex (2 with mutations but conflictive prediction depending on allele calling DB) confirmed by Sanger sequencing and Hain tests
Deeplex®-MycTB deployment and use

- Application on high performance, highly secured cloud-based platform, by early 2017
  - End-to-end integration from sequencers to computing centers to end-users PC/tablet (analysis/report) and smartphone (report)
  - Private supercomputer datacenters, with local data processing in major countries in Europe, Asia, and US; fully compliant with European and US regulation
  - Target: max. 1 hour/NGS run
- Profiled as first-line diagnostic test, but depending on drug resistance incidence, may be used after e.g. Xpert-based triage
- High-performance test most appropriate in regional, centralized laboratories
EDCTP2 H2020 “DIAMA” Clinical Trial (2016 on):

• 9 African + 4 EU partners
• Deeplex MycTB vs phenotypic, SNP-based molecular tests and patient outcomes
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Deeplex®-MycTB: resistance target coverage depth

- Target coverage depth of several 100-folds to several 1000-folds
- Bacterial load dependent