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Evaluation of Deeplex Myc-TB assay against Whole Genome Sequencing for MTBC lineage determination

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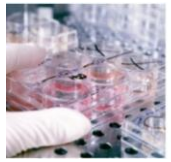
Division of the National Health Laboratory Service



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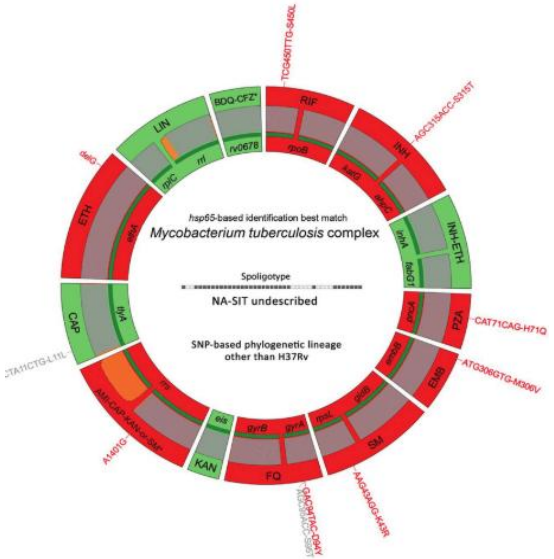
Background



- The Deeplex Myc-TB assay
  - WHO-recommended tNGS technology
  - Direct-from-sputum analysis (no culture required)
- The Deeplex Myc-TB assay has been integrated into South Africa's national care algorithms for managing DR-TB
- Comprehensive Profiling:
  - **Resistance prediction**: predicts resistance for up to 15 anti-TB drugs
  - **Lineage Identification**: *Maps MTBC lineages and sub-lineages.*
    - **Dual-Method Mapping**: Combines CRISPR locus mapping with SNP identification

Rationale of the study

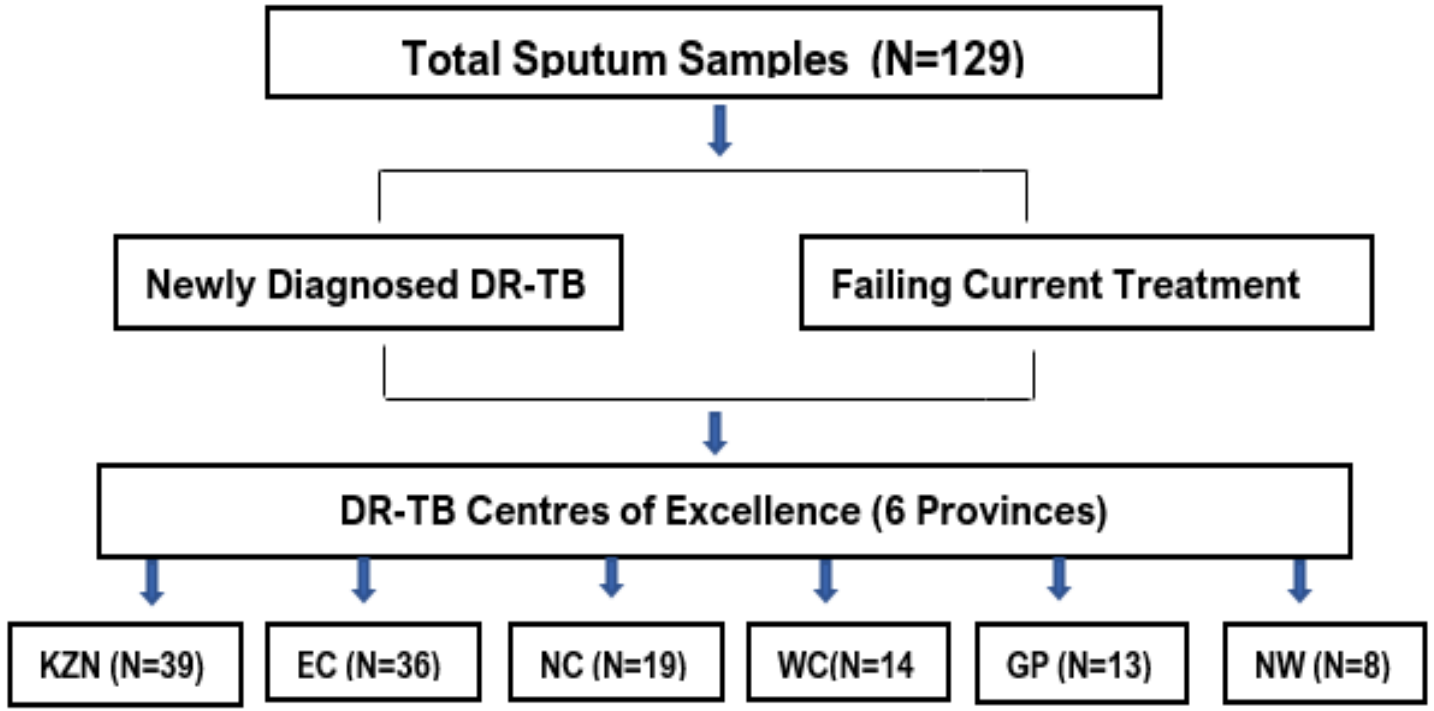
- The Deeplex Myc-TB assay has been mainly validated for predicting drug resistance, however its ability to identify MTBC lineages remains under-evaluated.
- Given that lineages directly influence virulence, transmission, and resistance patterns, accurate identification is crucial for effective public health interventions.

Aim

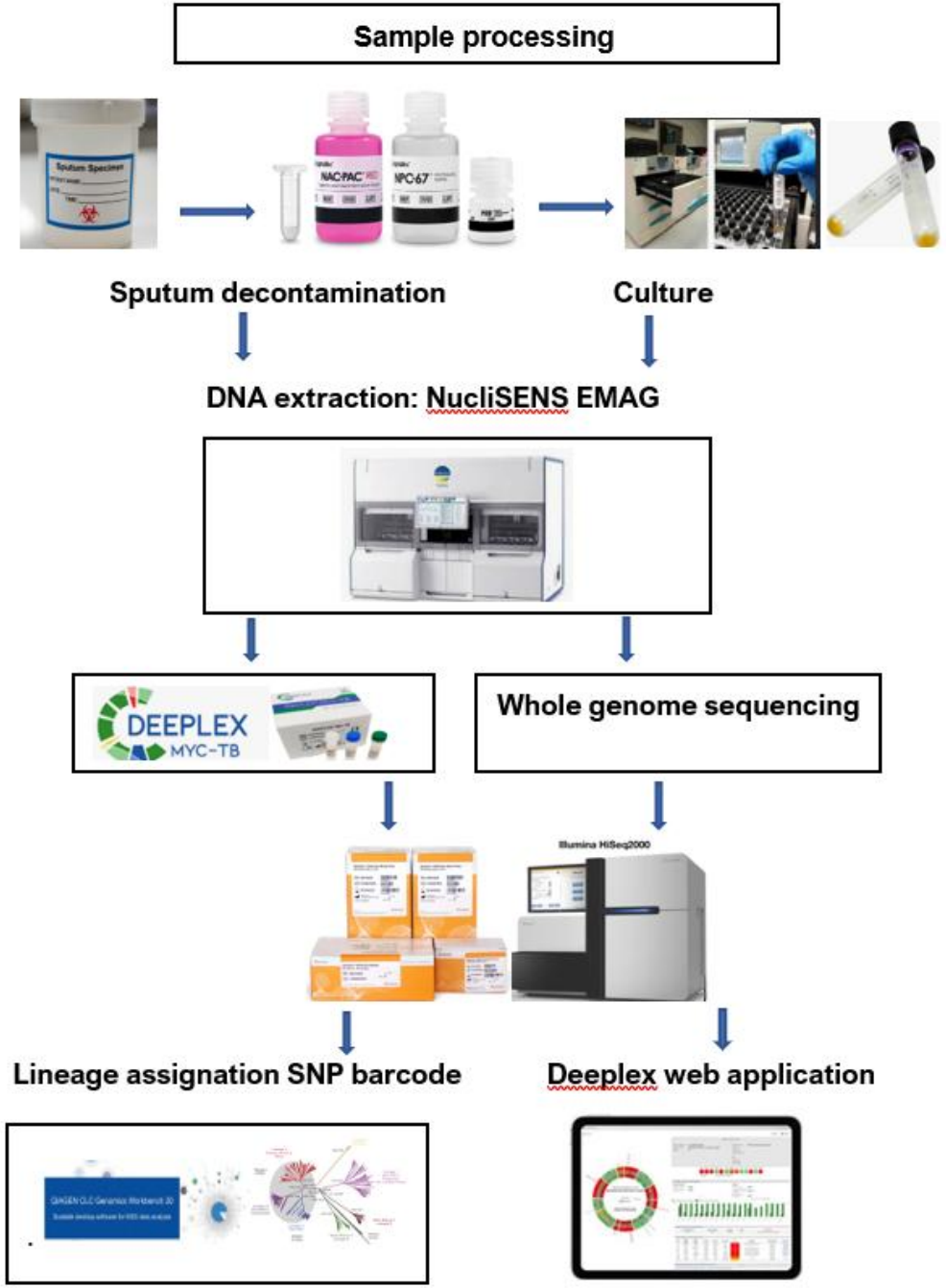
- To compare Deeplex Myc-TB assay against Whole Genome Sequencing (WGS) method to assess its accuracy in identifying MTBC lineages.

Methods

Study population



Methods


Sample processing
Sputum decontamination
Culture
DNA extraction: NucliSENS EMAG
DEEPLEX
MYC-TB
Whole genome sequencing
Illumina HiSeq2000
Lineage assignation SNP barcode
Deeplex web application

Results

Determination of MTBC lineages by WGS

Lineage Name	Sub-Lineage	L1	L2	L3	L4	Total
Indo-Oceanic	L1.2.2.1	1				1
	L1.2.2.2	1				1
East-Asian (Beijing)	L2.2		14			14
	L2.2.1		33			33
	L2.2.1.1		21			21
	L2.2.2		9			9
East-African-Indian	L3			1		1
	L3.1.1			4		4
	L3.1.2			1		1
Euro-American	L4.1				1	1
	L4.4.1.1				7	7
	L4.4.1.1.1				4	4
	L4.4.2				1	1
Euro-American (X-type)	L4.1.1.1				2	2
	L4.1.1.3				5	5
Euro-American (Haarlem)	L4.1.2.1				1	1
Euro-American (LAM)	L4.3.2.1				7	7
	L4.3.3				4	4
	L4.3.4.1				2	2
Euro-American (mainly T)	L4.8				2	2
	L4.8.1				2	2
Euro-American (H37Rv-like)	L4.9.1				1	1
Total		2	77	6	39	124

- 124 samples were analyzed
- 5 were excluded due to Deeplex assay failure to detect MTBC

Four main lineages were identified

- L1 (Indo-Oceanic) 2% (2/124): 2 sub lineages
- L2 (East Asian/Beijing) 62% (77/124): 4 sub lineages
- L3 (East-African-Indian) 5% (6/124): 2 sub lineages
- L4 (Euro-American) 31% (39/124): 13 sub lineages

Results

Determination of MTBC lineages by Deeplex Myc-TB assay

L (Deeplex)	Lineages						
	L1	L2	L3	L4	N/A	Mixed	Total
L1	1						1
L1,1.2.2	2						2
L2		66					66
L2: 3.67% L2: 96.33%		1					1
L3.1.1, 3.1.2.1			6				6
L4				8			8
L4: 1.27% L4.3: 98.73%				1			1
L4: 14.23% L4.3: 85.77%				1			1
L4: 6.84% L4.3: 93.16%				1			1
L4.3				4			4
L4.8: 1.81% L4 : 98.19%				1			1
Other than 4.9					29		29
Other than 4, other than 4.9: 6.25% L2: 93.75%						1	1
Other than 4.9: 3.03% L2: 96.97%						1	1
L4.1.1.1: 10.66% L2: 95.25%						1	1
Total	3	67	6	16	29	3	124

Four main lineages were identified

- L1: 2% (3/124): 1 sub lineages
- L2: 54% (67/124): no sub lineages
- L3: 5% (6/124): 2 sub lineages
- L4: 13% (16/124): 2 sub lineages
- Mixed of L2 and L4: 2.4% (3/124)
- 23.4% (29/124): categorised as "other than lineage 4.9"

Results

Comparison between WGS vs Deeplex Myc-TB assay

	L (Deeplex)						
L (WGS)	L1	L2	L3	L4	N/A	Mixed	Total
L1	2						2
L2	1	66		4	5	1	77
L3			6				6
L4		1		12	24	2	39
Total	3	67	6	16	29	3	124

Overall Concordance: 70% (87/124)

Lineage Performance:

- L1: 100% (2/2)
- L3: 100% (6/6)
- L2: 88% (66/77)
- L4: 31% (12/39)

Discordance was mainly due to: "Other than lineage 4.9" category

- 62% (24/39) of L4 cases
- 46% (5/11) of L2 cases

Results

Family	SIT (In silico Spoligotyping)	L1	L2	L3	L4	Total
EAI1_SOM	48	1	1			2
EAI1_SOM	806	1				1
CAS1_KILI	21			4		4
BEIJING	1		59		1	60
BEIJING	190		1			1
BEIJING	265		1			1
BEIJING-LIKE	796		3			3
LAM3 and S /convergent	4				1	1
LAM1	20				2	2
LAM3	33		1		4	5
LAM4	213		1			1
LAM4	60		1		4	5
LAM9	1176		1			1
T1	53		1		4	5
T1_RUS2	280				1	1
S	34		1		9	10
S	71		1		1	2
H3	50				1	1
X3	92				2	2
N/A	2016				1	1
N/A	2286				1	1
N/A	n/a		5	2	7	14
Total		2	77	6	39	124

- 89% (110/124) of the isolates into 22 shared international types (SIT),
- 11% (14/124) could not be categorised (N/A)
- WGS vs. Spoligotyping: 80.6% agreement (100/124)
- Discordance was mainly due to
  - No SIT designation available (67%, (16/24)
  - SITs not found in SITVIT (SIT-2016 and SIT-2286)

Discussion

- **Lower assay resolution:** the Deeplex assay demonstrated lower resolution than WGS.
- **Lineage concordance:**
  - SNP-based analysis showed 70% overall concordance
  - In silico spoligotyping achieved higher agreement at 81%
  - Deeplex performed poorly on L4 isolates at just 31%
- **Primary drivers of discordance**
  - Other than lineage 4.9 classification by Deeplex assay
    - Accounted for 62% of L4
- **Sub-lineage assignment**: Deeplex showed lower sub-lineage resolution than WGS.
  - Often fails to assign specific sub-lineages once the main lineage is identified.
  - This aligns with prior published abstract reporting missing sub-lineage data for about **two-thirds** of samples

- **Mixed infection:** Deeplex detected mixed infections in 3 samples that were not detected by WGS, discrepancies are likely due to
  - Deeplex assay higher sensitivity, software misclassification, or contamination.
  - Consistent with previous study, Deeplex showed higher mixed infections (6.7%) than WGS MICK-MAGMA (1.7%).
- **Sample failure:** The Deeplex failed to detect MTBC in 5 samples that were successfully identified by WGS
- Discrepancies between methods likely due to differences in sample input or bacterial concentration,
  - **Input differences:** WGS (culture) vs Deeplex assay (sputum).
  - **Low bacterial load:** 4 were smear-negative and 1 was graded 1+, the template DNA was likely below the minimum detection threshold for the Deeplex PCR-based amplification process

Conclusion

- **Deeplex limitations**: Provides lineage info but lacks WGS resolution, particularly for L4
- Failed to assign sub-lineages for most samples where lineage were identified.
- **High unclassified rate:** Categorized 23% of isolates as "other than lineage 4.9"
- **Limited public health utility**: The current assay format limits its effectiveness for public health interventions
- **Recommendation:** Lineage-specific markers must be refined to improve accuracy
- WGS remains necessary to achieve high-resolution phylogenetic analysis