

Implementation of DST Techniques in High-incidence, Low-income Countries

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Central Reference TB Laboratory

- Apex of a pyramidal structure of a TB laboratory network
- Population: 1 to 10 million or even more
- Specialized TB mycobacteriology laboratory handling high concentrations of viable Risk Group 3 pathogens



Central Reference TB Laboratory (Cont.)

- **Strengthening of operational and biosafety programmes above those for basic laboratory require major additions in:**
 - 1. Code of practice**
 - 2. Laboratory design and facilities**
 - 3. Laboratory equipment**
 - 4. Health and medical surveillance**
 - 5. Staff training and retention plans**
- **A Biosafety Level 2 plus or Level 3**



Summary of Biosafety Level Requirements

	BIOSAFETY LEVEL			
	1	2	3	4
Functional isolation of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation:				
-inward airflow	No	Desirable	Yes	Yes
-controlled ventilating system	No	Desirable	Yes	Yes
-HEPA-filtered air exhaust	No	No	Yes/No	Yes
Double-door entry	No	No	Yes	Yes
Anteroom	No	No	Yes	-
Autoclave:				
-on site	No	Desirable	Yes	Yes
-in laboratory room	No	No	Desirable	Yes
-double-ended	No	No	Desirable	Yes
Biological safety cabinets	No	Desirable	Yes	Yes
Personnel safety monitoring capability	No	No	Desirable	Yes

Laboratory biosafety manual. – 3rd ed., WHO 2004



Central Laboratory Functions

- Development of policy and standards
- Analysis of information for management
- Coordination of **training** in microscopy **and culture**
- **Education and training in biosafety**
- Supervision and quality assurance
- **Culture and identification of Mycobacteria**
- **Drug susceptibility testing (DST)**
- Preparation and distribution of reagents
- Technical control and repair services
- **Research** activities, **applicability of new techniques**



Central Reference TB Laboratory (Cont.)

To be organized:

- Supply and maintenance
- Reliable, regular, safe specimen transport
- Rapid, effective communication of results
- Integrated internal QC and external QA
- Link to a SRL will be obligatory for EQA of DST

With NTP:

- Criteria to request for/to perform culture, DST
- Staffing, training and funding



Type of DST

Phenotypic tests (various modifications as indirect /direct version)

- Comparison of growth or metabolism (general or more specific) on/in plain and drug containing media
- „Critical“ drug concentration or comparison with “wild type” control
- Problems: dependent on viability and/or similar growth on both media

Genotypic tests

- Detection of genetic mutations conferring drug resistance
- Advantage: Inactivated specimen can be sent/used
- Problems: multiple genes, unknown mutations, silent mutations (best: rpoB gene; sensitivity >95%)
- Cross-contamination



Training Topics

- Drug powder quality, storage & expiry
- Correct drug concentration: **Potency!**
- Drug dissolution and dilution
- Medium preparation (heating) and storage
- Internal quality control, H37Rv
- Reading: 4 weeks / six weeks

Preparation of suspension



- Inoculum size (10 μ l vs 100 μ l), dispersion & dilution



SOP Format and Management

NATA German Nepal T.B. Project Kalimati Kathmandu Head: Dr. Bhawana Shrestha	Standard Operating Procedure (SOP)	Version: 04
	Preparation of Drug LJ Medium	Date: 29.08.2003 Page : 1 of 9 Code: TB - 11.01

DHSM, Factor 1.277: Sol. I: 12.77 mg dissolved in 250 ml dist. water **(40 µg/ml)**
Sol. II: 10 ml **Sol. I** add up to 20 ml with dist. water **(20 µg/ml)**

Final concentration in drug media (µg/ml):

	4 µg/ml	2 µg/ml	1 µg/ml	0.5 µg/ml
Media (ml)	180	18.0	18.0	18.0
Sol. I (ml)	20	-	-	-
Sol. II (ml)	-	2.0	1.0	0.5
Water (ml)	-	-	1.0	1.5
Final volume (ml)	200	20	20	20



GENETUP Laboratory, Kathmandu

- Performs culture and DST, proportion method on LJ since 1987
- Passed all EQCs since then successfully
- More than 15,000 specimen are tested for at least the four first line drugs
- Participated in all four DST round of WHO/IUATLD
- Participated in Study A and Study C of the Union
- Performs DST for SLD since 2003 on LJ
- All laboratory investigations for DOTS Plus programme (> 290 patients)



Liquid media

Middlebrook 7H-9 broth used for identification tests as well as for primary isolation. Related broth 7H-12B and 7H-13A are commercially available in systems with indicators for growth.

- These systems reduce the time to detection to 50%
- The detection rate is increased by 6-10%
- DST for FLD and some SLD will shorten the turn around time
- The contamination rate increases by a factor of 2-5



High-Tech Meets Ancient Technique



Rapid culture and DST, 1st and (2nd) line drugs

1. Safe laboratory
2. Well trained staff
3. Agar media to detect contaminants
4. Maintenance, support
5. Stable electricity?



Lessons learned and Recommendations

➤ Procurement

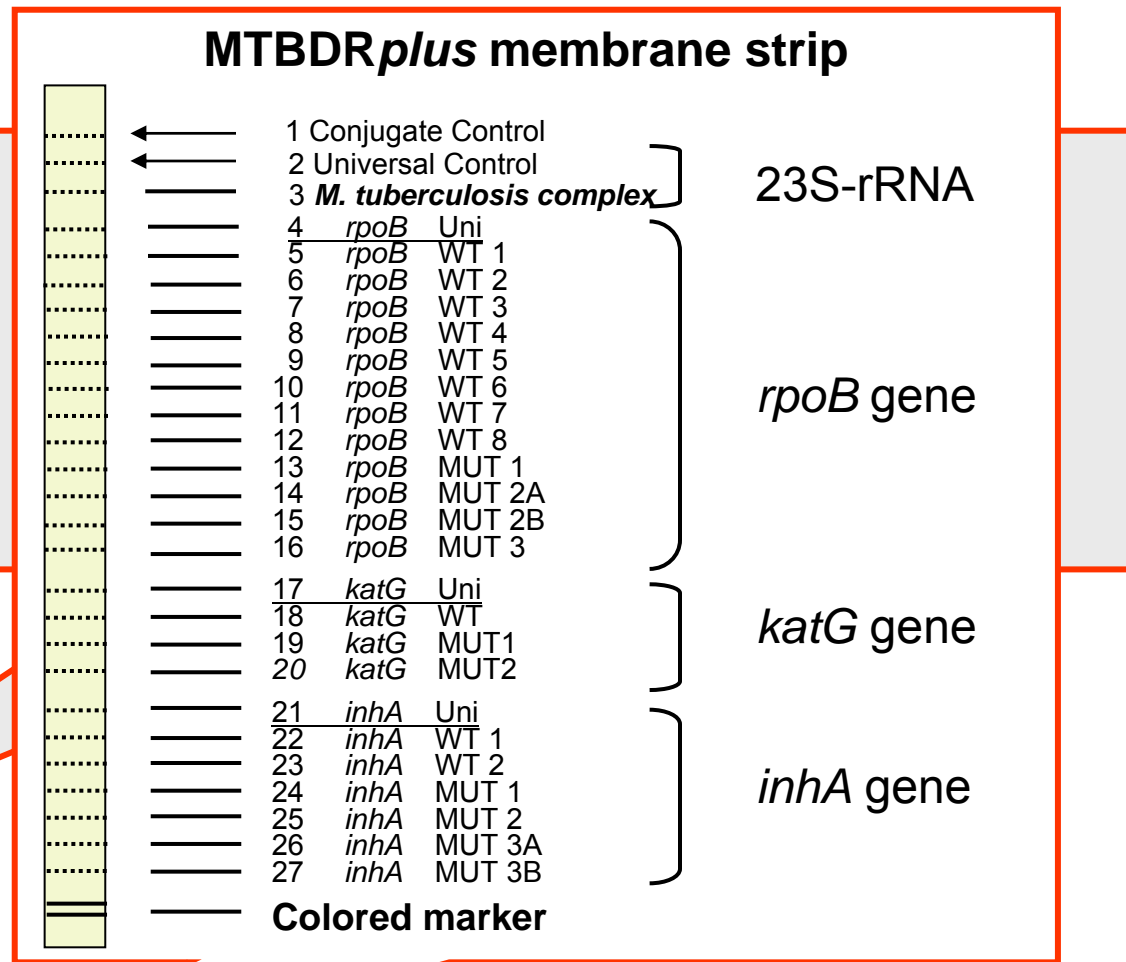
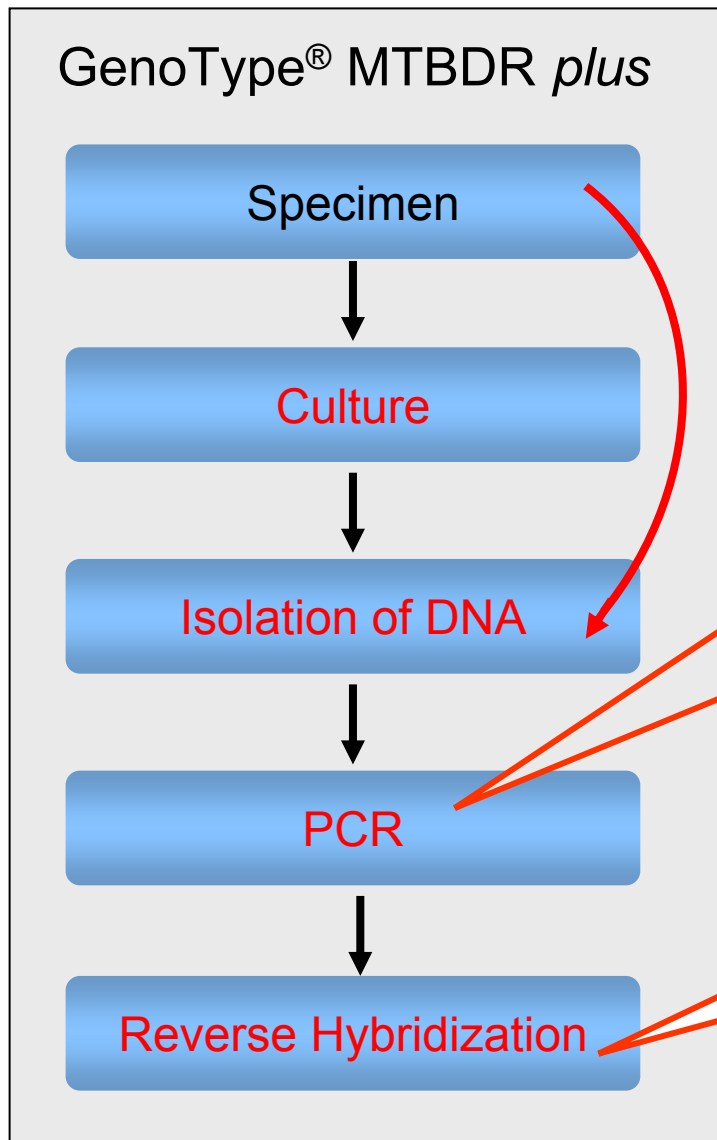
- **Delays** due to delivery, shipment, customs clearance have to be considered
- Some reagents have **short shelf-lives** (about half a year) and may have even shorter expiry dates on arrival. Therefore **inventory management** and **ordering system** needs attention to avoid disruption

➤ DST

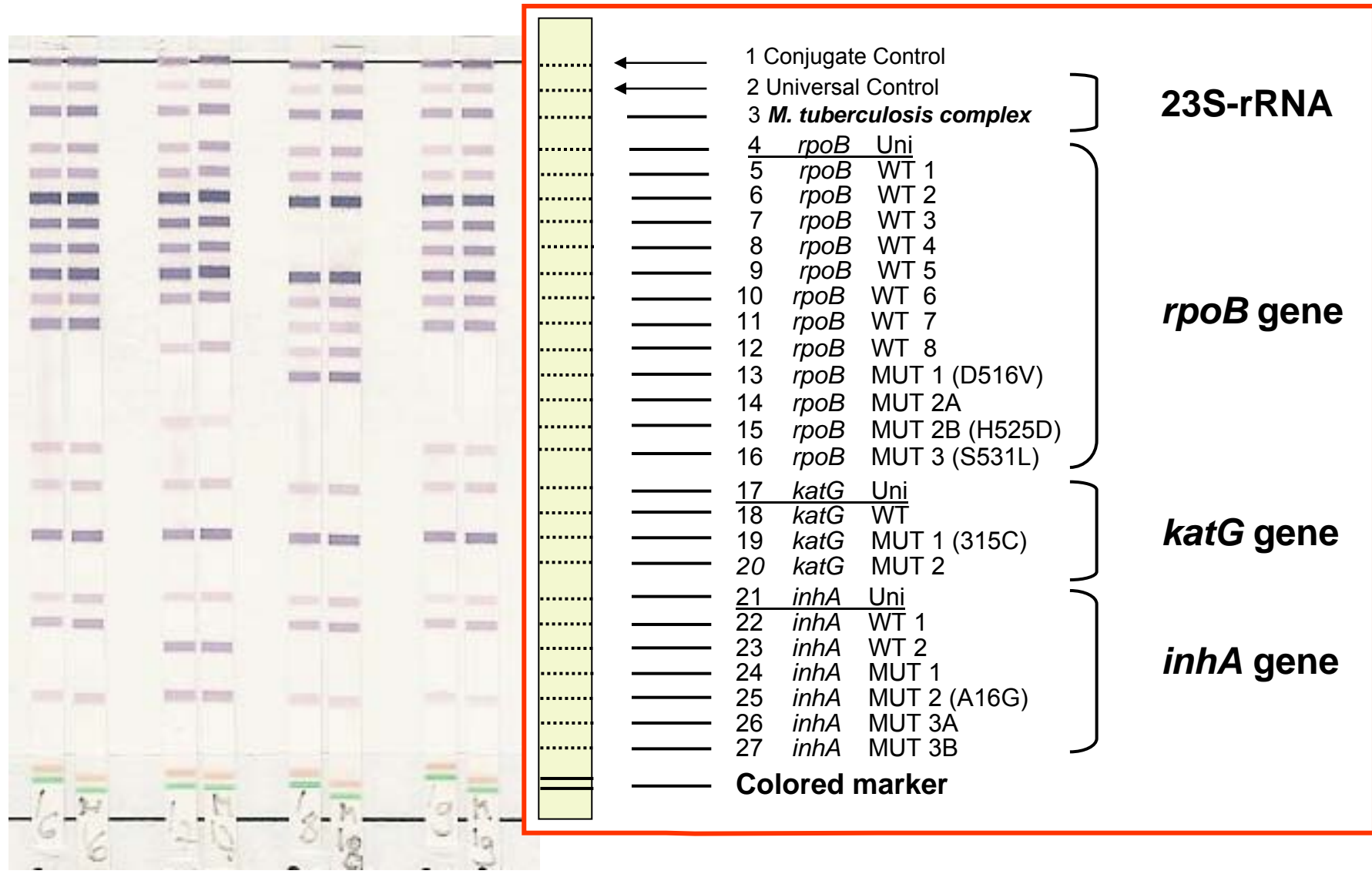
- Special training on “clean” working
- At present all positive tubes are checked for additional contamination

➤ Species identification (Genotypic tests)





GenoType[®] MTBDR*plus*



DST for DOTS Plus Program, Nepal

Proportion Method vs GenoType MTBDR Plus

Based on lab investigations of 297 patients' specimens

7 NTM (3 *M. fortuitum*/3 *M. intracellulare*/1 *M. abscessus*, identified with GenoType CM/AS)

Evaluated with both DST-systems: 272 patients

Total MDR TB cases: 248 = 91,2%

Detected MDR by PM: 98,0%

Detected MDR by GT: 91,9%



Comparison of results of DOTS Plus Program, Nepal

Test system	LJ/MGIT		GenoType	
Drug	RMP	INH	RMP	INH
false susc.	4	3	5 WT	23WT
false res.	1	0	0	0
correct susc.	22	14	23	14
correct res.	245	255	244	235
Total	272	272	272	272



katG/inhA Gene

Out of all for INH correctly resistant tested stains

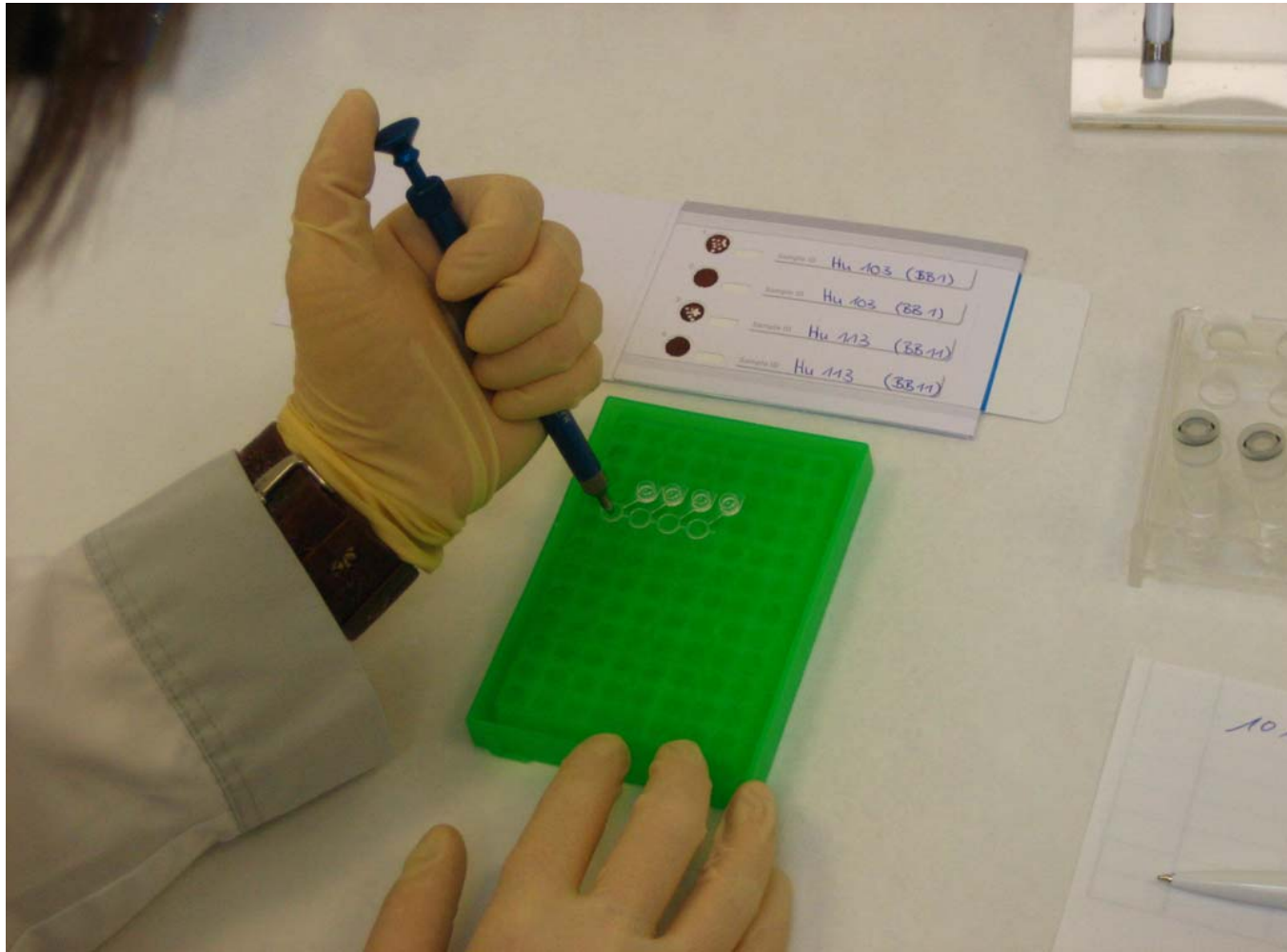
- **katG** gene showed in 83.4% of the cases mutations in codon 315
- **inhA** gene showed in 17.9% mutations in position 15 and one in position 16

Mutations in both region were found for 1.3% of strains

PTH resistance for inhA mutated strains were found for all strains when tested on LJ (28), but not using MGIT-system with 5µg/ml (recommendation in the last comparative study)



The DNA-transfer from the GenoCard®



Specimens on Filter Paper

- Washed sediment lysed in 100µl water, (15 µl), No 61
- Sediment after Petroff-decontamination lysed in 300µl water, (16 µl), No 62
- Sediment (20µl) directly, No 51
- Smear directly
 - Heat inactivated, No 51
 - Alcohol inactivated, No 51
 - Sediment directly, No 51



Steps to Improve Sensitivity

62 Filters (GenoCards) from Nepal

- Alkaline lysis of cells
 - No inhibition – good yield
- Punch size used 1mm Ø vs 2mm Ø
 - Equivalent results from 47 samples
 - Out of 15 samples 4 of 1mm and 11 of 2mm exhibited improved amplification
- No detectable “carry over” by the punching procedure
- Mixed hybridization patterns are a challenge for interpretation. Super-infection? Developing a resistance?



Summary

- Conventional DST (proportion method on LJ) for First (and Second) Line Drugs can be implemented with reliable results, but sustained external input is needed
- Genomic Tests are learned easily with some extra training on the use of equipment and waste handling to avoid cross contamination
- The use of liquid media caused more problems
 - Contamination rate for culture – **solved**
 - Occasional contamination in DST tubes – **under investigation**
 - Maintenance and technical support – **(2-3 days)**
 - Trouble shooting – by e-mail and on the spot **(4 times a year)**
 - Adjustment of SOPs - **ongoing**
- DST using the old “hand method” would solve at least the problems associated with running the instrument permanently

