THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

Training on New and rapid Tuberculosis diagnostics (first and second line Probe Assay)

Module 12: Interpretation of results-1st and 2nd line

Content outline

- Principle of result interpretation
- Role of various bands
- Prediction of resistance/susceptibility
- Genes for susceptibility/resistance to various drugs
- Differenciation between version 1 and version 2 second line LPA kit
- Discussion/ observing of some LPA strips/worksheets.

Group exercise-5 minutes

1. What drugs do the following genes of LPA represent:

   gyrA, Inh A, gyrB, rpoB, rrs, KatG and eis

2. What are the names in full and significance of the following bands on the LPA strip:

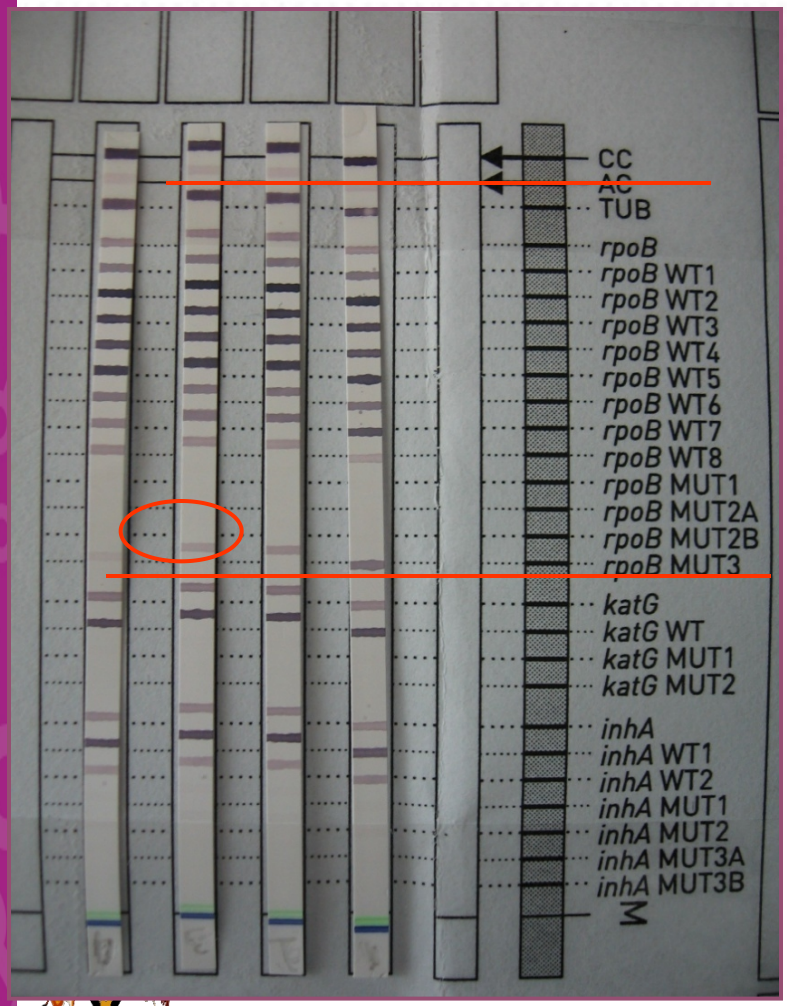
   CC

   AC

   TUB

3. Explain 2 reasons why LPA results may be un-interpretable or invalid

Principle of interpretation of results



- Align the bands Conjugate Control (CC) and Amplification Control (AC) on each strip with the respective lines on the worksheet
- Tape each strip onto the corresponding line on the worksheet
- Determine the band positivity and positions on each strip using the reference reading chart of the kit and mark the results on the worksheet
- Bands should only be considered positive if they are approximately as strong as the AC control
- NB: 1st and 2nd line strips should only be taped to the corresponding worksheets since the strips have different length.

CC band

CC band (conjugate control)

- The Conjugate Control (CC) line must be present
- If CC is negative, the conjugation or substrate reaction was unsuccessful, either due to error in the procedure or due to problems with the reagen

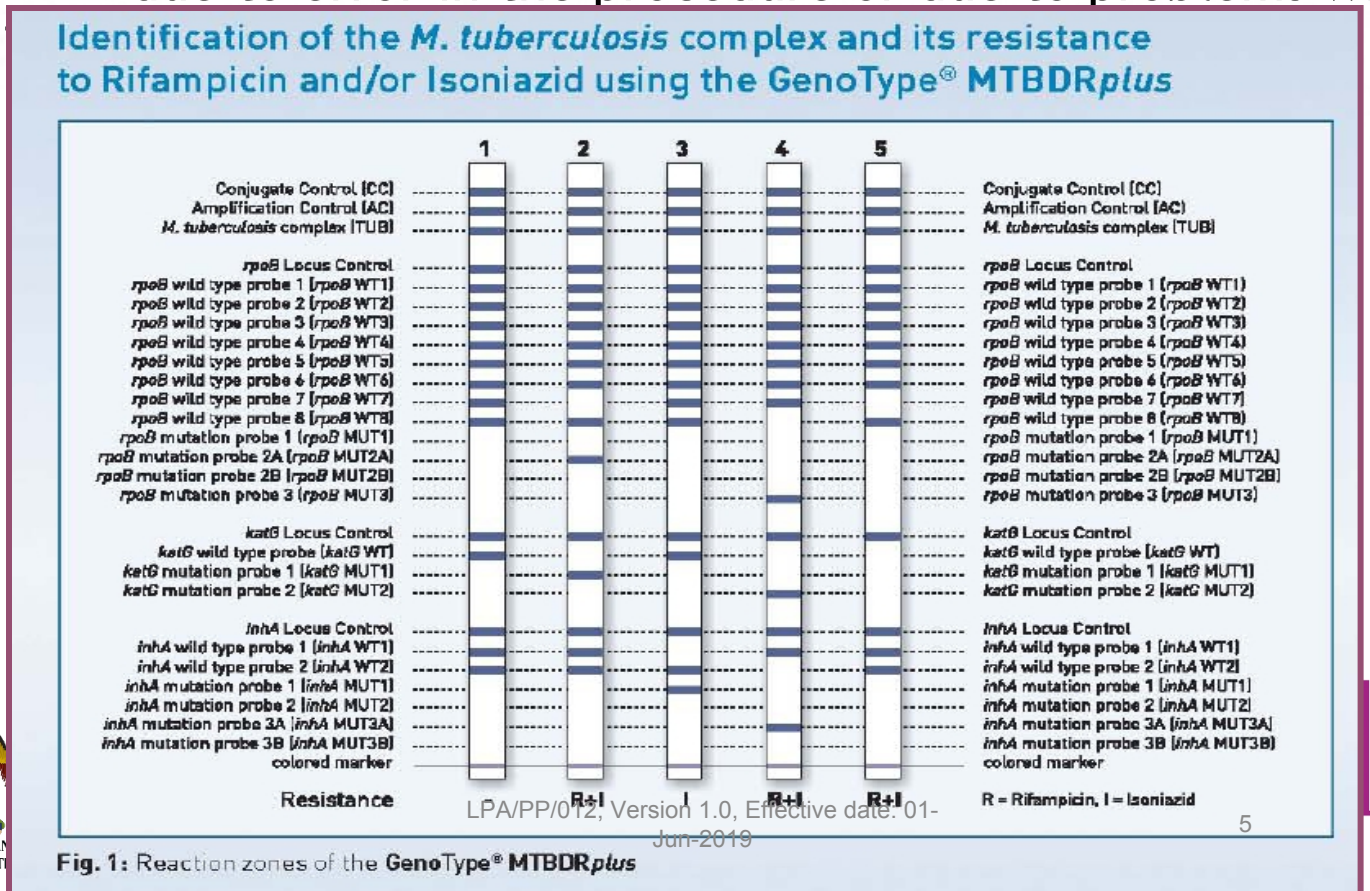


Fig. 1: Reaction zones of the GenoType® MTBDRplus

AC band

AC band (Amplification control)

- The Amplification Control (AC) line detects an internal control in PNM (Primer Nucleotide Mix) that is specific for all currently known mycobacteria as well as Gram$^+$ bacteria of high G+C content. Therefore, this control is not mycobacteria specific!

- If AC is positive, errors during extraction and amplification set-up and presence of amplification inhibitors in the specimen can be excluded

- Signal of AC can be weak or even absent while results for other bands (TUB, rpoB, katG , inhA, *gyrA*, *Inh A*, *gyrB*, *rpoB*, *rrs*, *KatG and eis* locus controls) may be positive. This might be due to competitive reactions between AC and the other locus controls during the amplification. In this case, the strip can be evaluated.

AC band-2

- A weak or missing AC band with negative test result for TUB, rpoB, katG , inhA, *gyrA*, *Inh A*, *gyrB*, *rpoB*, *rrs*, *KatG and eis* locus controls may indicate potential mistakes during extraction and amplification set-up, or the presence of amplification inhibitors; in this case, the test has to be invalidated

- All bands (except of CC) should be compared to the AC control band for density

- Bands that are less dense than the AC band should not be reported

- The AC band always has to be positive even in negative controls, indicating that the amplification reaction was proper and not inhibited

TUB band

- A positive *M. tuberculosis* control (TUB) band indicates the presence of members of the *M. tuberculosis* complex

- If the TUB zone is negative, the tested bacterium does not belong to the *M. tuberculosis* complex; therefore, the presence or absence of any other bands (except CC and AC) cannot be considered for evaluation and the result is reported as MTBC not detected

Locus control zones

- This is the first band for each of the locus genes: rpoB, katG , inhA, *gyrA, Inh A, gyrB, rpoB, rrs, KatG and eis.*
- Locus control zones detect a gene region specific for their respective genes

These locus control bands must always be present for the assay to be considered valid for the corresponding target.

However, when only one gene locus control band is missing, the results for the other genes for which the gene locus control band is present can be interpreted.

Prediction of Resistance not detected

- Only those bands whose intensities are about as strong as (or stronger than) that of the AC are to be considered
- Wild type probes encompass common resistance-associated mutation sites of the respective genes
- When all wild type probes of a gene stain are positive, there is no detectable mutation within the examined regions; the tested strain may be considered sensitive for the respective antibiotic

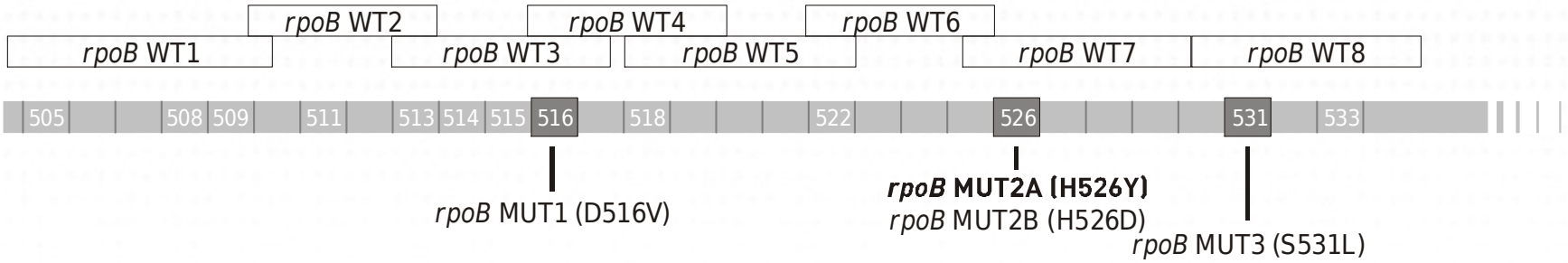
Prediction of Resistance

- In case of mutation, the respective amplicon cannot bind to the corresponding wild type capture probe on the strip due to the mismatch
- Positive hybridization signal with a mutation-specific capture probe (for common mutations only!) may predict resistance to the respective antibiotic *directly*
- The absence of a signal for at least one of the wild type probes may predict resistance to the respective antibiotic *indirectly*

Prediction of Resistance Inferred

- Incase one or more WT probes in regions of the gene known to confer resistance to the drug are not developed, and none of the MUT probes in the corresponding region is developed, then the result is termed as resistance inferred.

ifampicin resistance: *rpoB* ene



- rpoB wild type probes: WT 1 to WT 8
- rpoB mutation-specific probes: MUT D516V, H526Y, H526D, S531L

Detection of mutations:

- absence of wild type signals
- presence of common mutation-specific signals

High-level Isoniazid resistance: *katG* gene

Mutations in *katG* and the corresponding wild type and mutation probes

missing wild type probe	analysed codon	mutation probe	mutation
katG WT	315	katG MUT1 katG MUT2	S315T1 S315T2

Resistance is reported Mutations associated with high level increase in MIC

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Low-level Isoniazid resistance: *inhA* gene

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Mutations in the *inhA* promotor region and the corresponding wild type and mutation probes

missing wild type probe	analyzed nucleic acid position	mutation probe	mutation
inhA WT1	−15	*inhA* MUT1	C15T
	−16	*inhA* MUT2	A16G
inhA WT2	−8	*inhA* MUT3A	T8C
		inhA MUT3B	T8A

Resistance is reported as Mutation associated with low level increase in MIC

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Mutations in the *gyrA* gene and the corresponding wild type and mutation bands

Failing wild type band	Developing mutation band	Mutation	Phenotypic resistance
gyrA WT1	-	G88A	
		G88C	
gyrA WT2	*gyrA* MUT1	A90V	
	gyrA MUT2	S91P	
gyrA WT3	*gyrA* MUT3A	D94A	FLQ
	gyrA MUT3B	D94N	
		D94Y	
	gyrA MUT3C	D94G	
	gyrA MUT3D	D94H[1)]	

Mutations in the *gyrB* gene and the corresponding wild type and mutation bands

Table 2: Mutations in the *gyrB* gene and the corresponding wild type and mutation bands

Failing wild type band	Developing mutation band	Mutation[1]	Phenotypic resistance
gyrB WT	*gyrB* MUT1	N538D	FLQ
	gyrB MUT2	E540V	

Mutations in the *rrs* gene and the corresponding wild type and mutation

Failing wild type band	Analyzed nucleic acid position	Developing mutation band	Mutation	Phenotypic resistance				See figure 1
rrs WT1	1401	*rrs* MUT1	A1401G	KAN	AMK	CAP		example 2 and 6
	1402	-	C1402T	KAN		CAP	VIO	example 3
rrs WT2	1484	*rrs* MUT2	G1484T	KAN	AMK	CAP	VIO	example 4

KAN, kanamycin; AMK, amikacin; CAP, capreomycin; VIO, viomycin

Mutations in the *eiss* gene and the corresponding wild type and mutation bands

Failing wild type band	Developing mutation band	Mutation	Phenotypic resistance
eis WT1	-	G-37T	low-level KAN
eis WT2	*eis* MUT1	C-14T	
	-	C-12T	
	-	G-10A	
eis WT3	-	C-2A	

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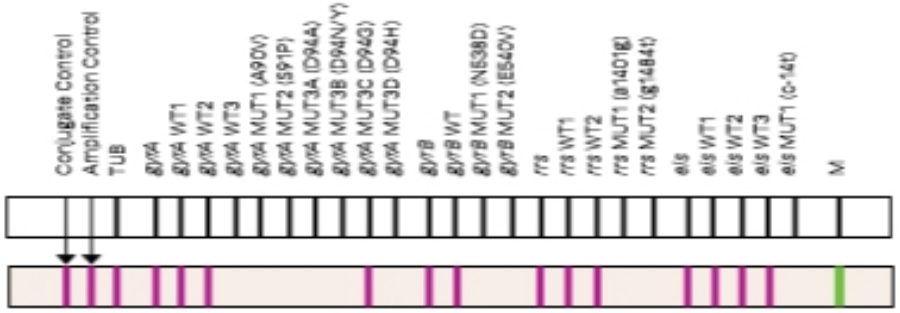
Case Scenario 1



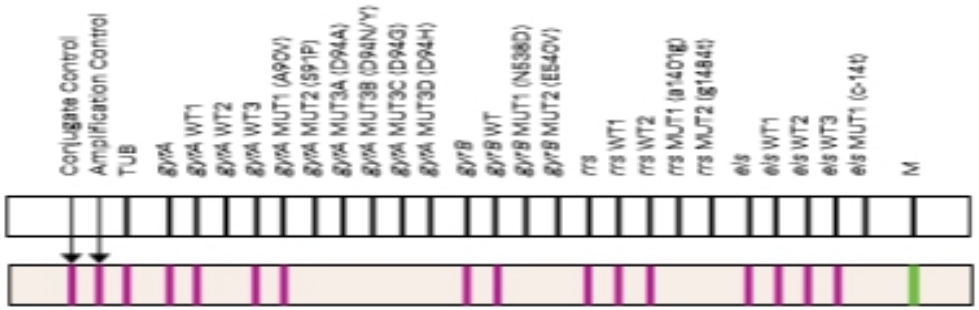
If one of the following MUT probe is developed:

- gyrA MUT3C
- gyrA MUT3D
- gyrA MUT3B

Genotypic report: Levofloxacin: Resistance detected

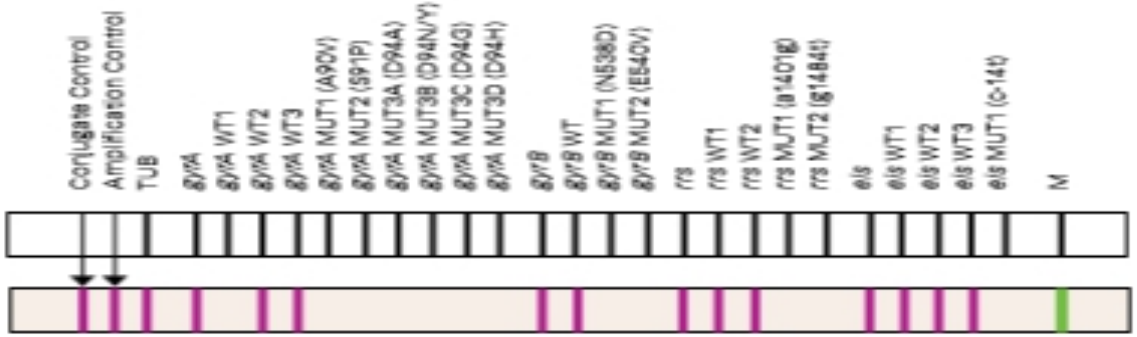
Moxifloxacin: Mutation associated with high-level increase in MIC for Mfx detected

Case Scenario 2



If one of the following MUT probe is developed: • gyrA MUT1
• gyrA MUT2
• gyrA MUT3A
• gyrB MUT1
• gyrB MUT2
Genotypic report: Levofloxacin: Resistance detected.
Moxifloxacin: Mutation associated with at least low-level increase in MIC for Mfx detected

Case Scenario 3



If one of the following WT bands is not developed:

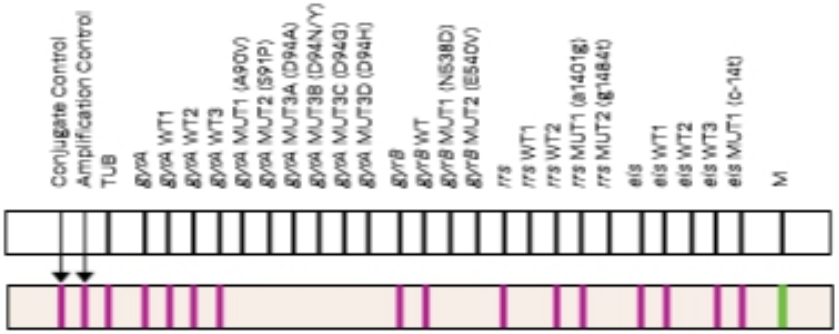
- gyrA WT1
- gyrA WT2
- gyrA WT3
- gyrB WT

and none of the MUT probe is developed in the gyrA and gyrB regions.

Genotypic report: Levofloxacin: Resistance inferred.

Moxifloxacin: Mutation associated with at least low-level increase in MIC for Mfx inferred.

Case Scenario 4



If one of the following MUT bands is developed:

- rrs MUT1
- rrs MUT2
- eis MUT1 (i.e. eis c-14t)

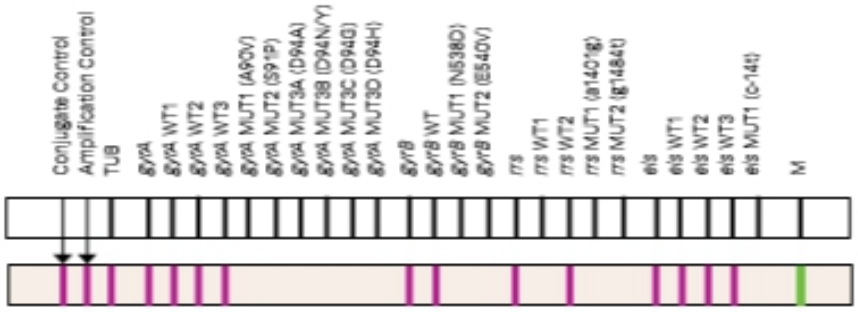
Genotypic report: In case of rrs mutations only or mutations in both rrs and eis:

- Amikacin: Resistance detected
- Kanamycin: Resistance detected
- Capreomycin: Resistance detected.

Case Scenario 4

- In case of eis mutation c-14t only:
- Amikacin: Resistance not detected
- Kanamycin: Resistance detected
- Capreomycin: Resistance not detected

Case scenario 5



If one of the following WT bands is not developed:

- rrs WT1
- rrs WT2

and none of the MUT probes is developed in the rrs region.

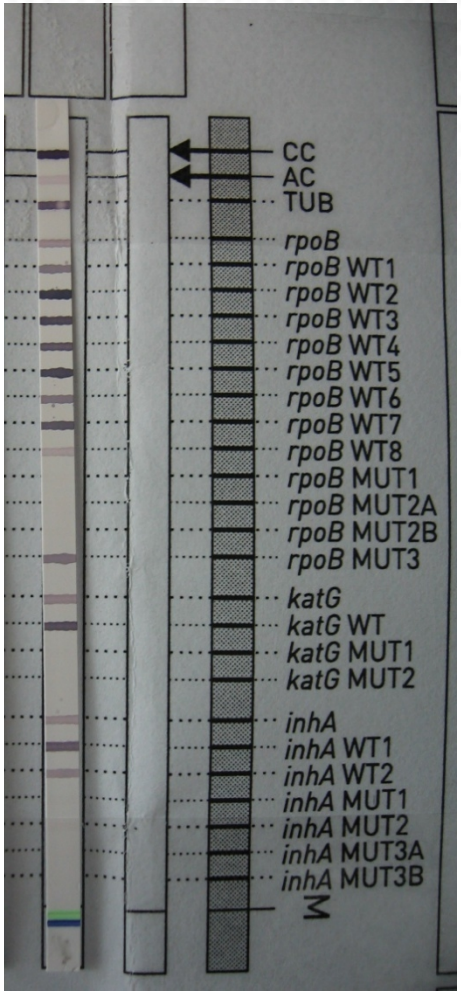
Genotypic report: Resistance inferred for

Kanamycin

Capreomycin

Amikacin

d pattern



- Hetero-resistance = equal representation of susceptible and resistant mutants of the same strain
- Mixed pattern = mutual presence of a resistant strain and a second, susceptible strain
- Not pure culture
- Carry-over contamination

Assessment

1. Does LPA confirm Mycobacteria species identification just like the xpert MTB/Rif assay?
2. What are the signs of susceptibility when interpreting LPA results.
3. What are the signs of resistance when interpreting LPA results.
4. What are the signs of cross contamination during interpretation of LPA results

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- Compare all molecular results with other laboratory results, i.e. culture, identification, conventional drug susceptibility testing and, if possible, with clinical findings.

- In the event of discrepant results-then repeat testing with another specimen from the same patient may be helpful.

- All bands (except CC) should be compared to the AC control band for density.

- Repeat the LPA assay: i) faint bands are gotten from the sediment-repeat with positive culture or ii) when the banding pattern is not clear for cultured specimens.

ences

- GenoType MTBDRplus product insert. Hain Lifescience. Version 2.0.
- Yasuhiko Suzuki et al 1998; Journal of Clinical Microbiology. P 1220-1225)
- GLI TB training package http://www.stoptb.org/wg/gli/trainingpackages.asp

Acknowledgments