# Hain GenoType Line Probe Assay: Overview and Training

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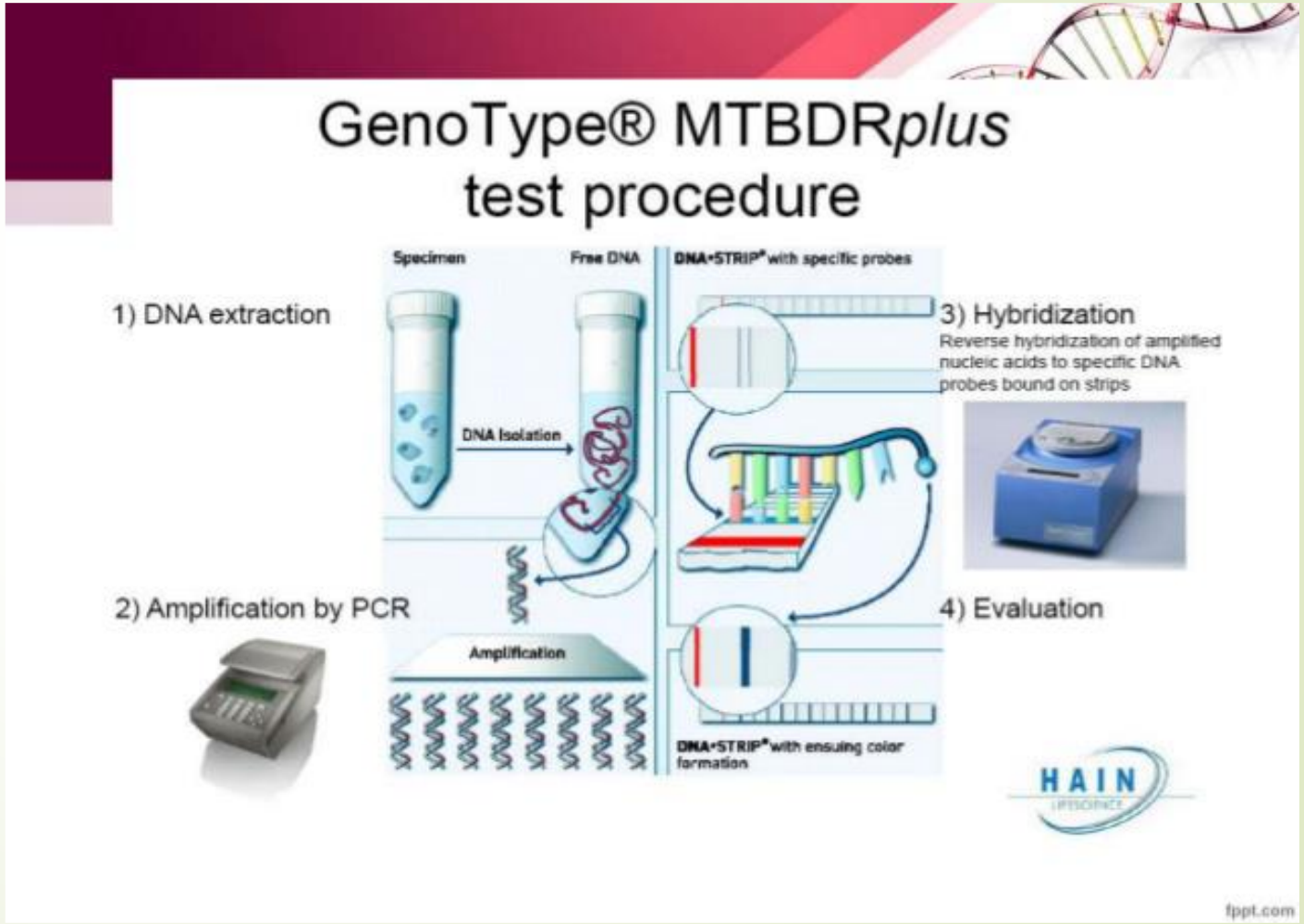
**TB DIAGNOSTICS WORKSHOP, 8-12 JULY 2019.**

# HAIN Genotype Line Probe Assay (LPA)

- The LPA uses PCR and reverse hybridization methods for rapid detection of mutations associated with drug resistance.
- The LPA is designed to identify MTBC and simultaneously detect mutations associated with drug resistance.
- The LPA is should be performed in laboratories with a proven capacity to conduct molecular testing including appropriate laboratory infrastructure and equipment.
- This must also include the necessary biosafety precautions and the prevention of contamination.

# LPA Procedures: Key Steps

- 1. DNA extraction a. Clinical pulmonary specimens (decontaminated) b. Cultured isolates (solid or liquid cultures)
- 2. Amplification
- 3. Reverse Hybridization
- 4. Analysis

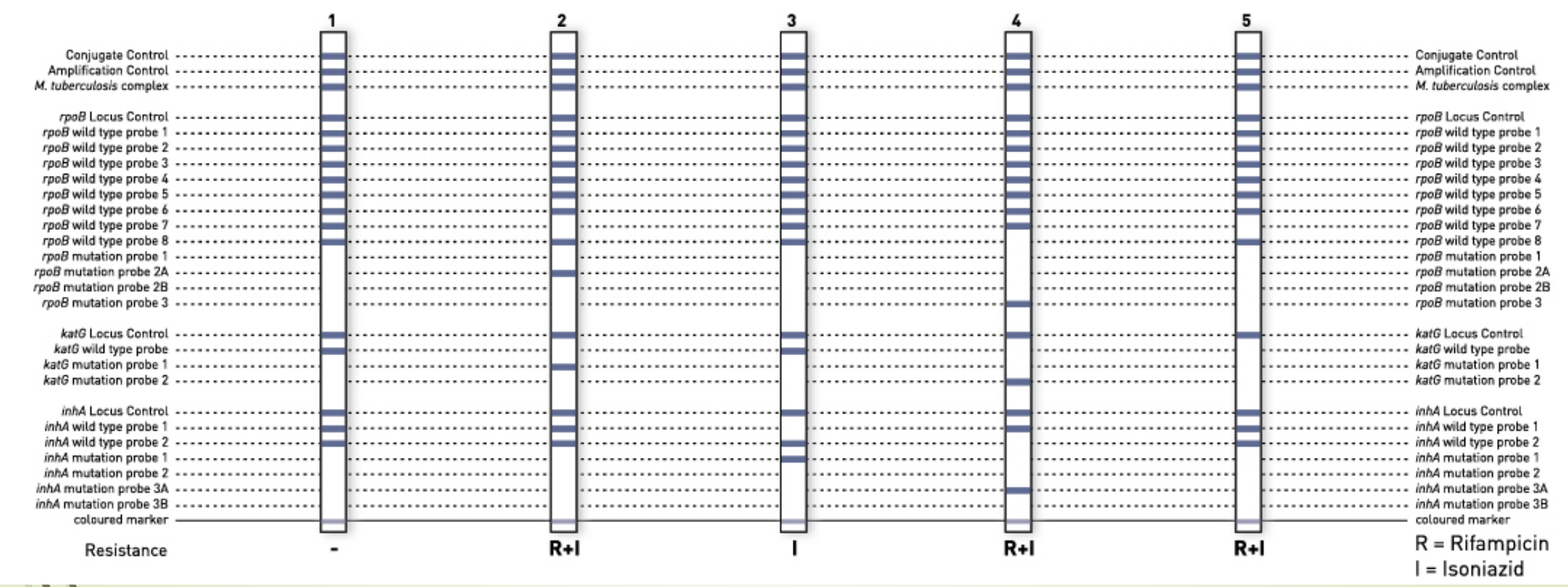


# LPA Test Controls

- Conjugate Control (CC) -Included on Strip
  - Demonstrates efficient conjugate binding and substrate reaction
  - Line must be present for a valid result
- Amplification Control (AC)-Included on Strip
  - Demonstrates successful amplification
- Negative Control (NC)-Recommended Test/Batch
  - Contains water instead of DNA to control for contamination
  - Only the CC and AC bands should be present on this strip

## MTBDRplus Assay: Tests for Mutations in INH and Rifampin

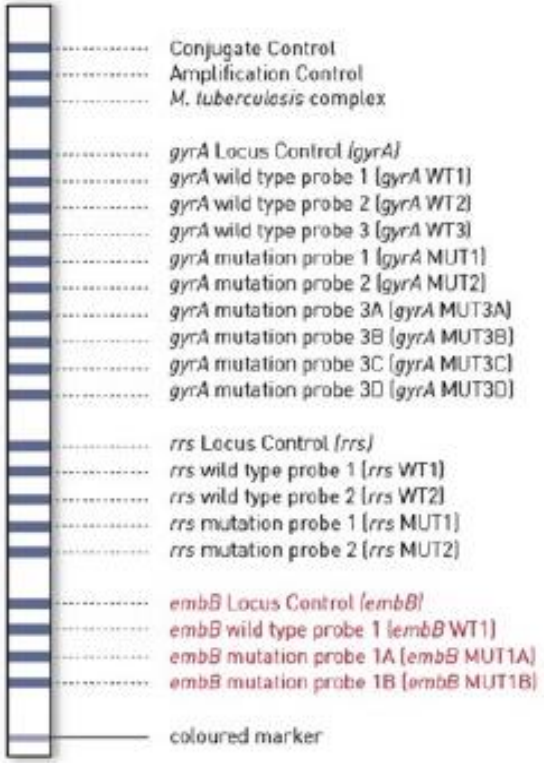
- rpoB: If mutation present, this may correlate with resistance to Rifampin
- inhA: If mutation present, this may correlate with resistance to INH (low level resistance)
- katG: If mutation present, this may correlate with resistance to INH (high level resistance)



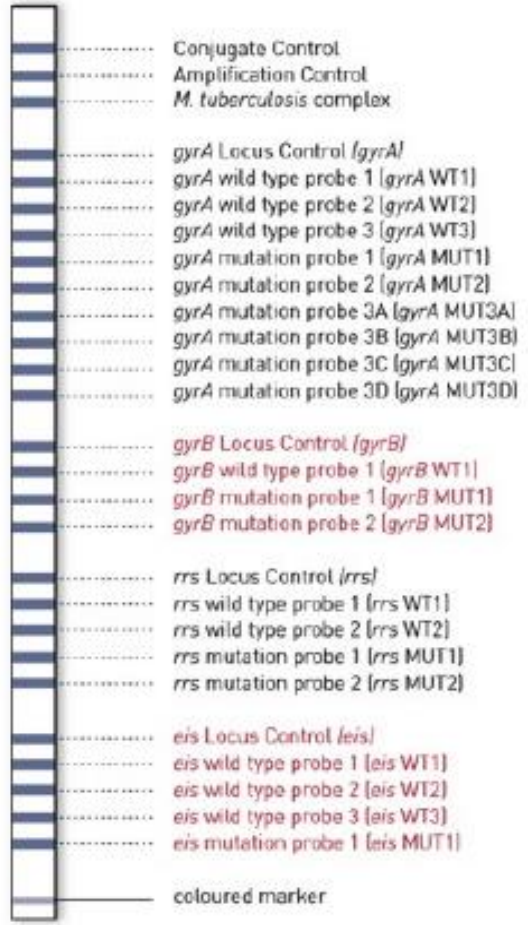
# MTBDRsl Assay: Tests for Mutations in Fluoroquinolones, Aminoglycosides and Ethambutol (Note differences in test versions!)

gyrA or gyrB: If mutation present, this may correlate with resistance to Fluoroquinolone (Ex: Ofloxacin, Moxifloxacin)
rrs/eis: If mutation present, this may correlate with resistance to Aminoglycosides (Amikacin, Kanamycin)
embB: If mutation present, this may correlate with resistance to Ethambutol

**GenoType MTBDR*sl* VER 1.0**




**GenoType MTBDR*sl* VER 2.0**



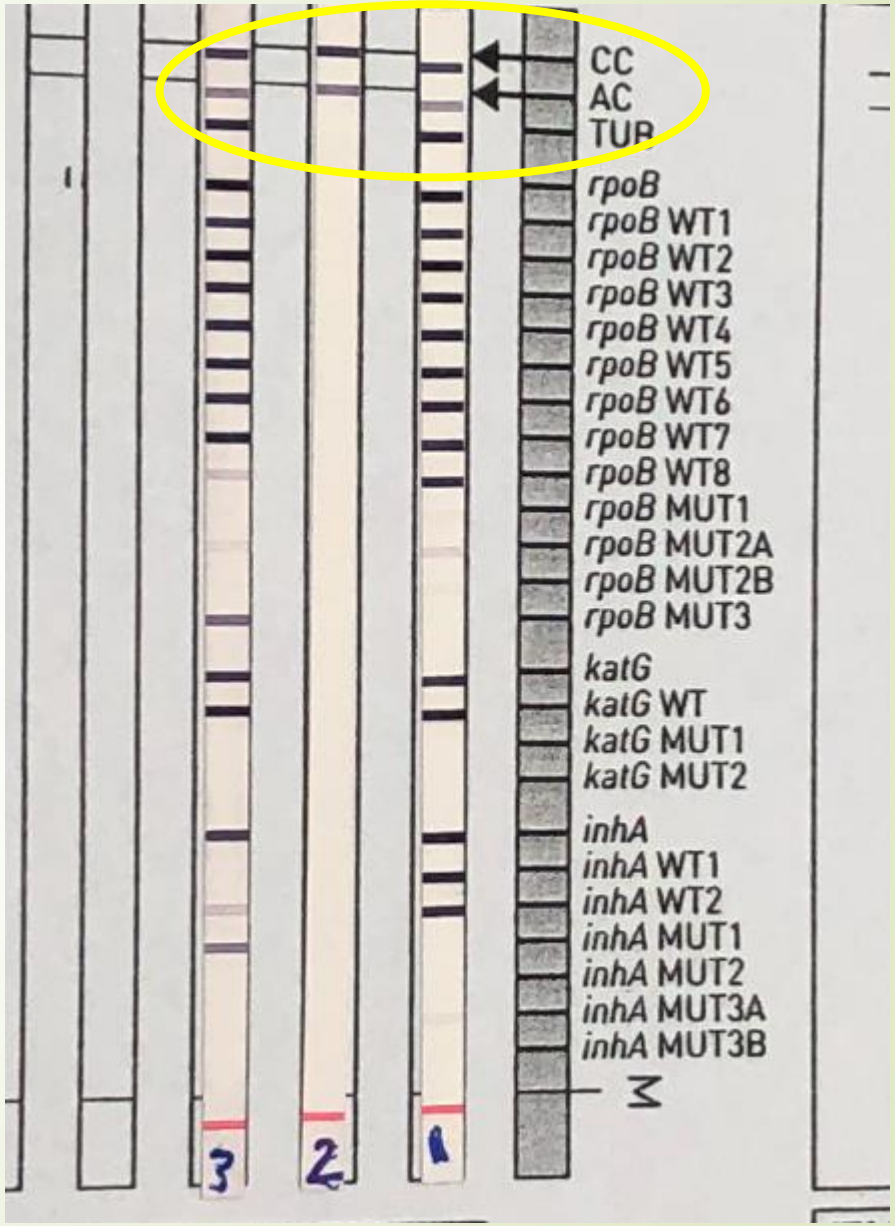

Differences between the two versions are marked in red

# Interpreting Line Probe Assay Strips

- **Step 1: Look at the "CC" band, "AC"band and "TUB" band.**

- **Conjugate Control (CC) Band:** This should be present for each test (if not, the test is invalid)
- **Amplification Control (AC) Band:** This band should be present for each test (if not, the test is invalid)

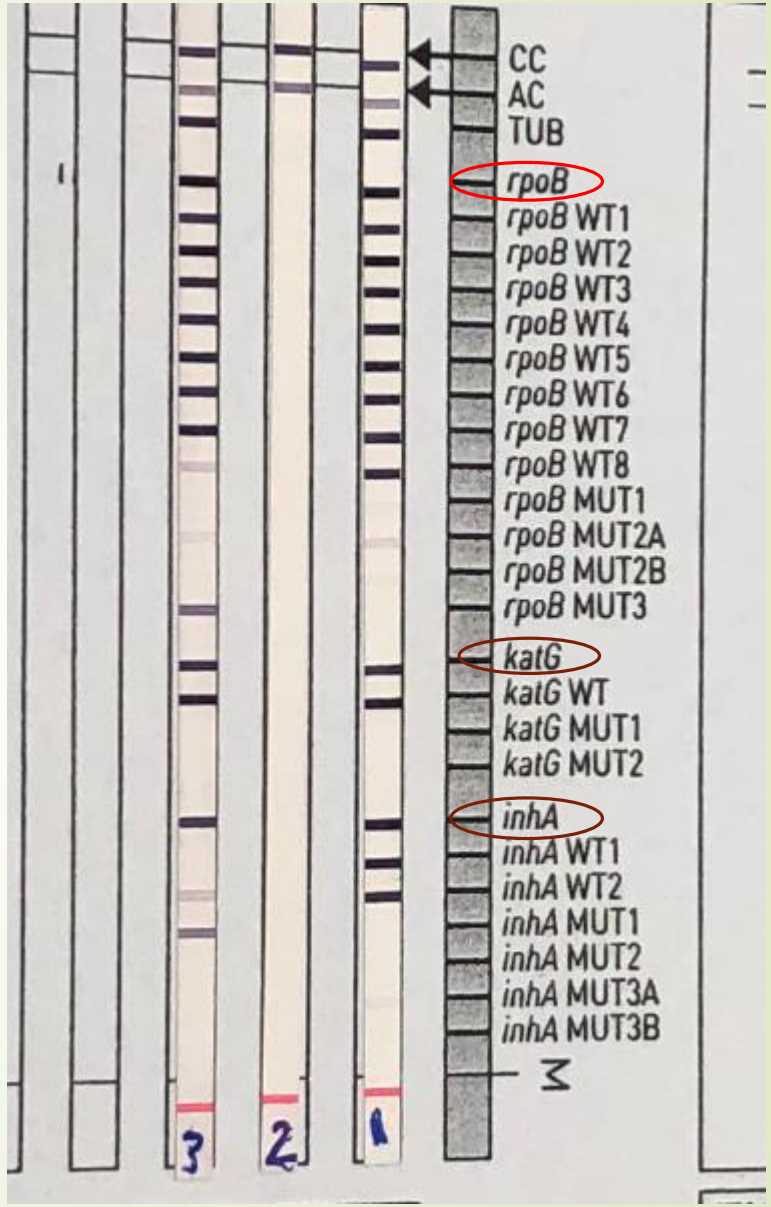
- ***M. tuberculosis* complex (TUB) Band:** This band should hybridize with all members of the MTB complex. A positive (+) test result shows the band present, while a negative (-) test result shows the band as absent.



# Interpreting Line Probe Assay Strips(2)

- **Step 2: Look at the drug control bands (called Locus controls, these are the non-WT or MUT bands)**

- For a valid test, there should be a band present for each of the drug control bands.
- **If the TUB band is present** (indicating an MTBC positive result) and the drug control band is absent, the results for that particular drug are indeterminate.
- **If the TUB band is absent** (indicating an MTBC negative result), there should be NO drug control bands present for that particular sample*.
- (***NOTE**: If TUB band is negative but there is still an evaluable susceptibility pattern, MTB complex is suspected, but test should be repeated)



# ssay Strips

## p 3: Look at the results of the "WT" and UT" bands (only interpret bands that are darker or rk as the AC band)

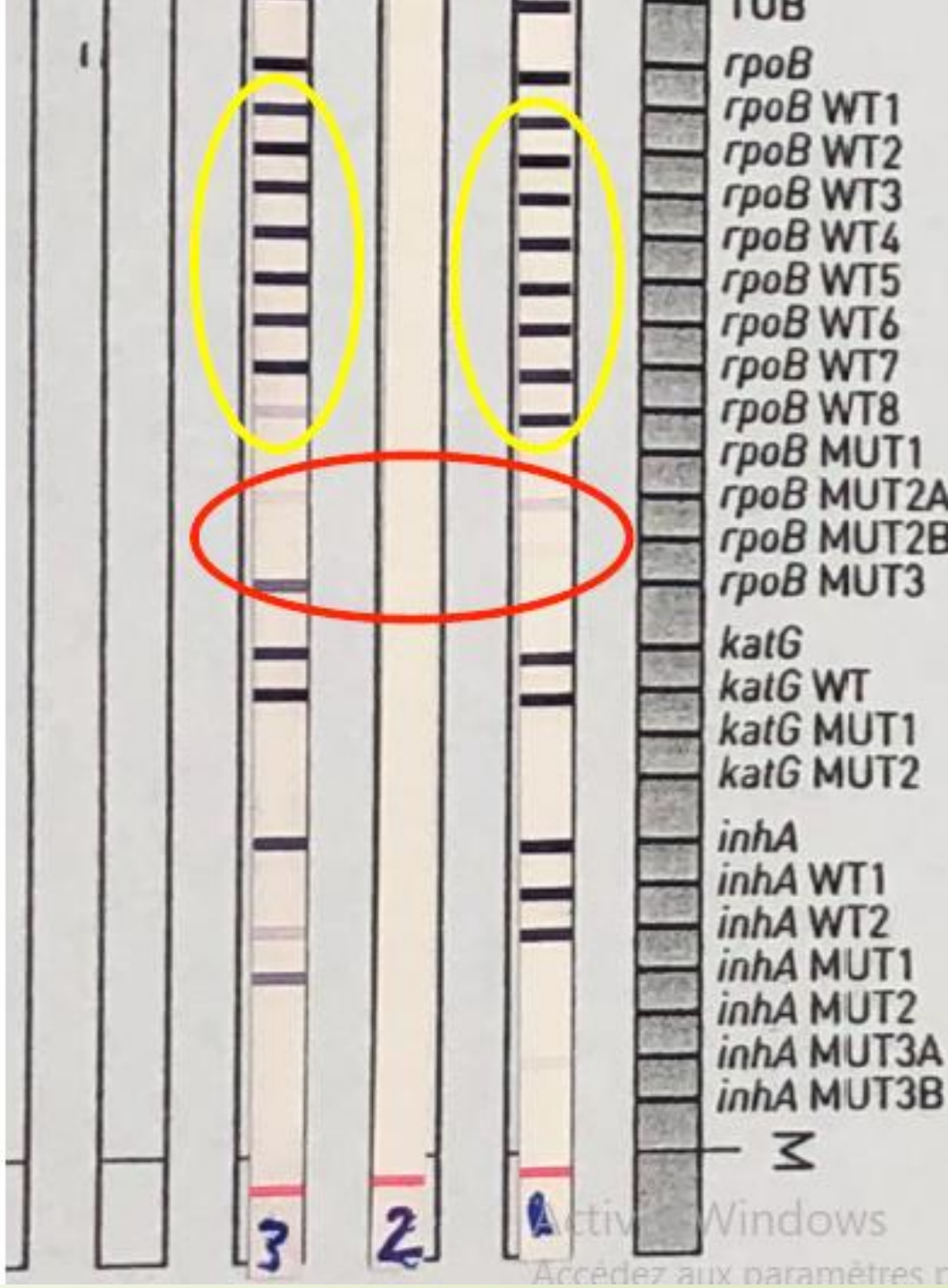
### Type (WT) probes:

s should be present for all WT probes for a specific drug e bands will appear weaker in intensity than others). ne yellow circle in the picture to the right.

WT bands are missing, this means there could be a tion that confers resistance.

### tion (MUT) probes:

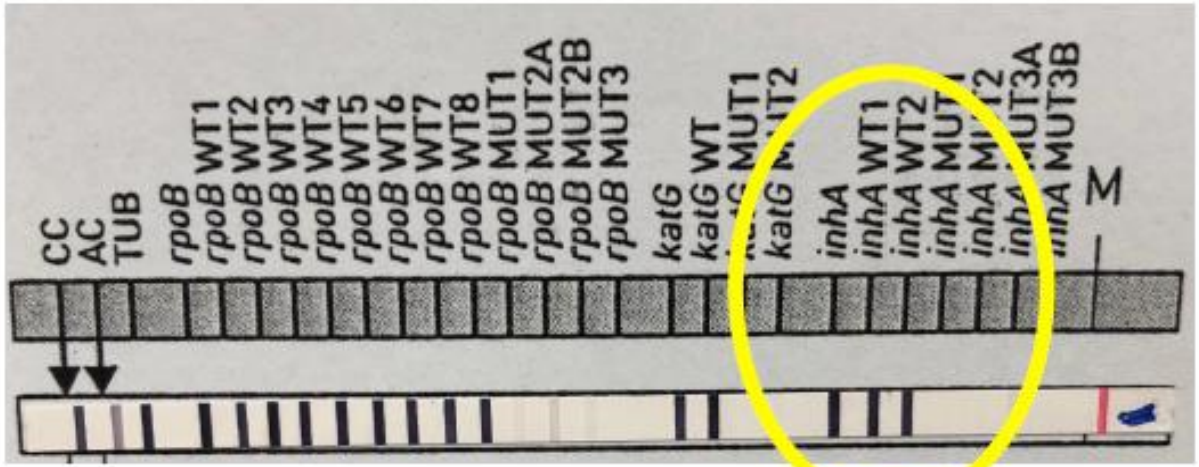
re is a band present (and it is darker than the AC ol), this means a mutation is present that could confer ance. See the red circle in the picture to the right, that s a clear rpoB mutation (MUT3) in Sample #3.



# Interpreting Line Probe Assay Strips: **A word of caution!**

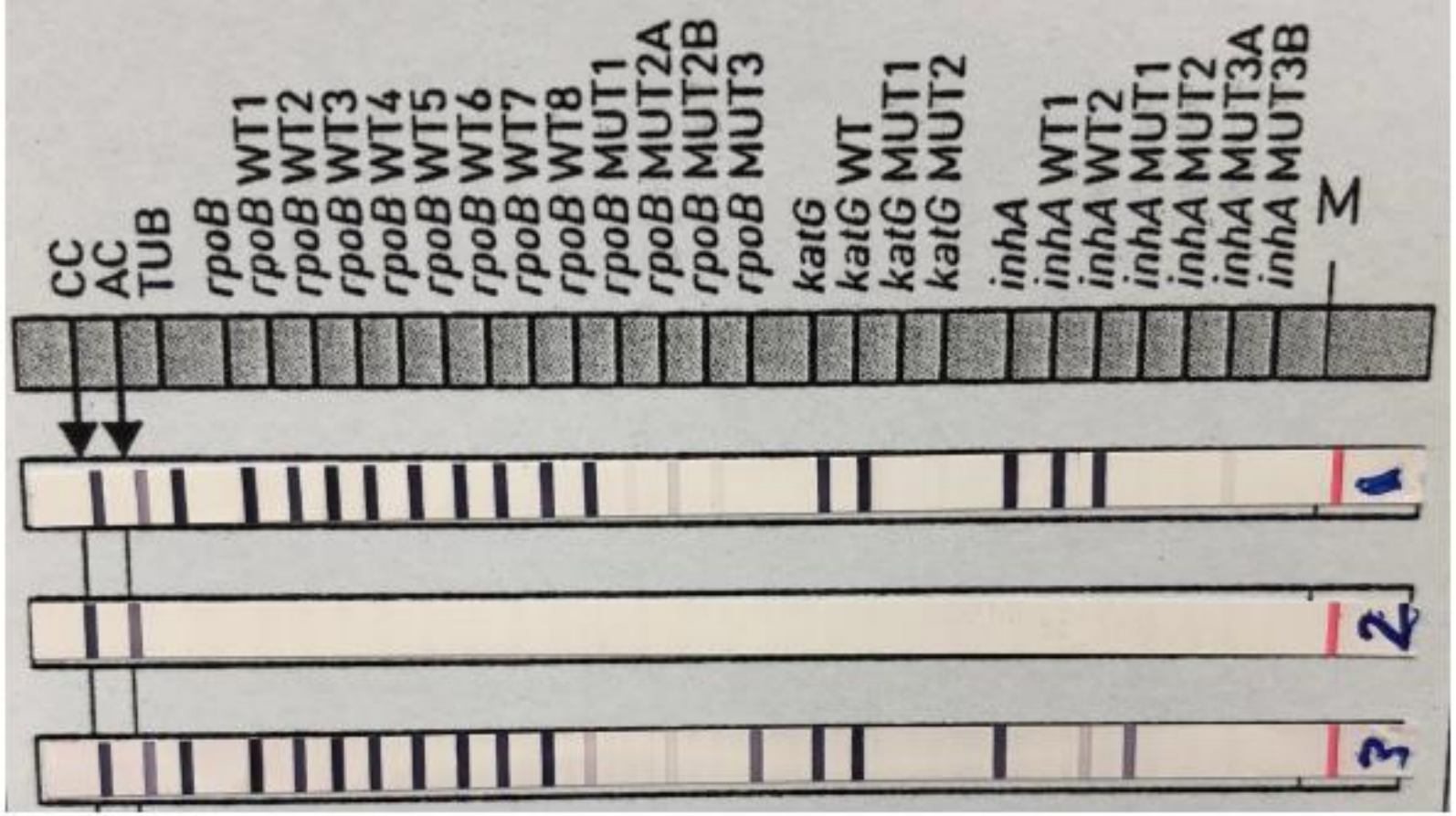
Make sure to align the strips on the sheet to correspond with the correct band location!

Otherwise this could make it more challenging for interpretation.




Strip alignment is correct, and inhA results can be matched to the banding patterns

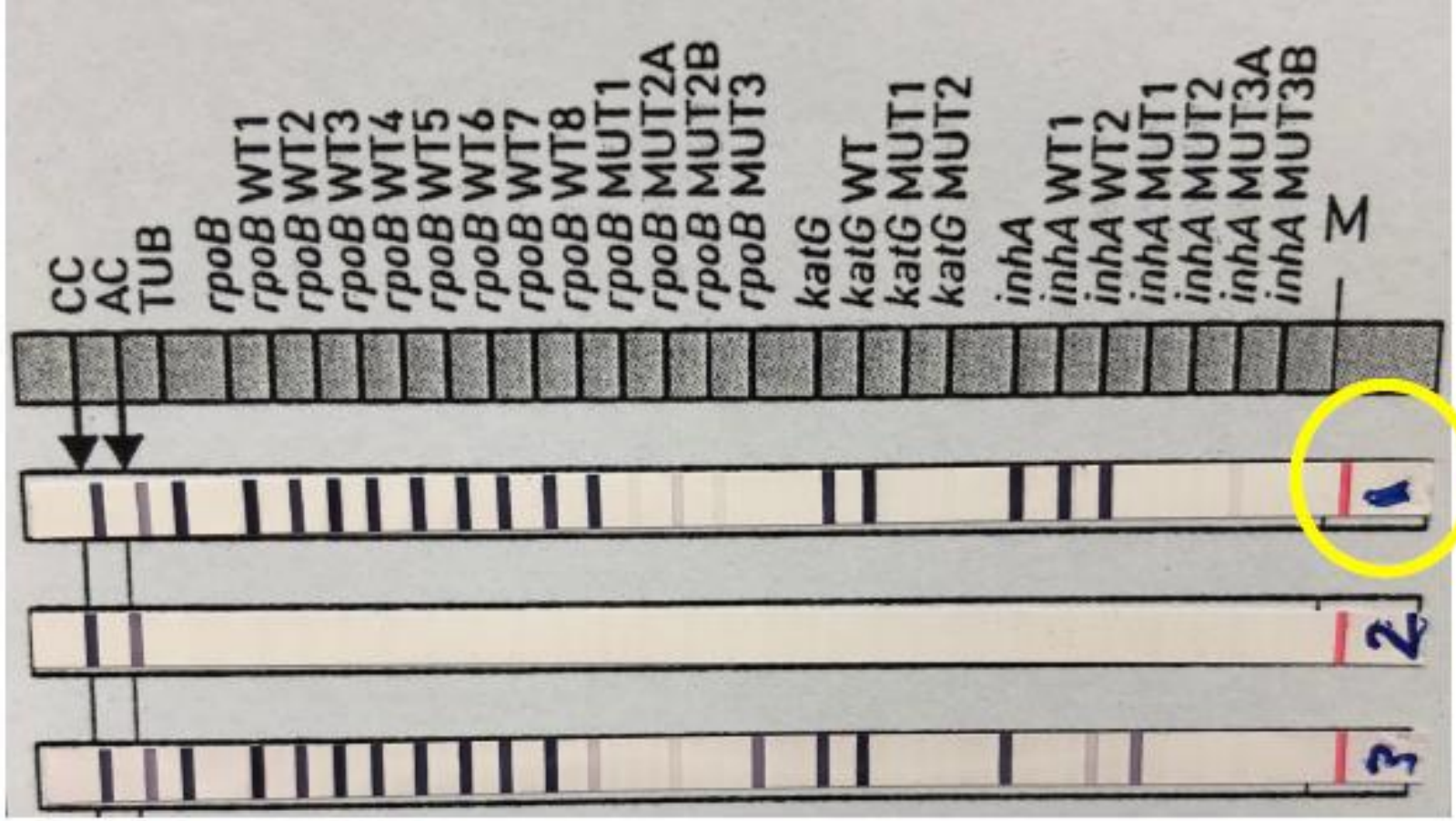

Strip alignment is slightly shifted, making inhA result interpretation a bit more difficult (The resulting bands preser here should indicate inhA and WT bands present only)



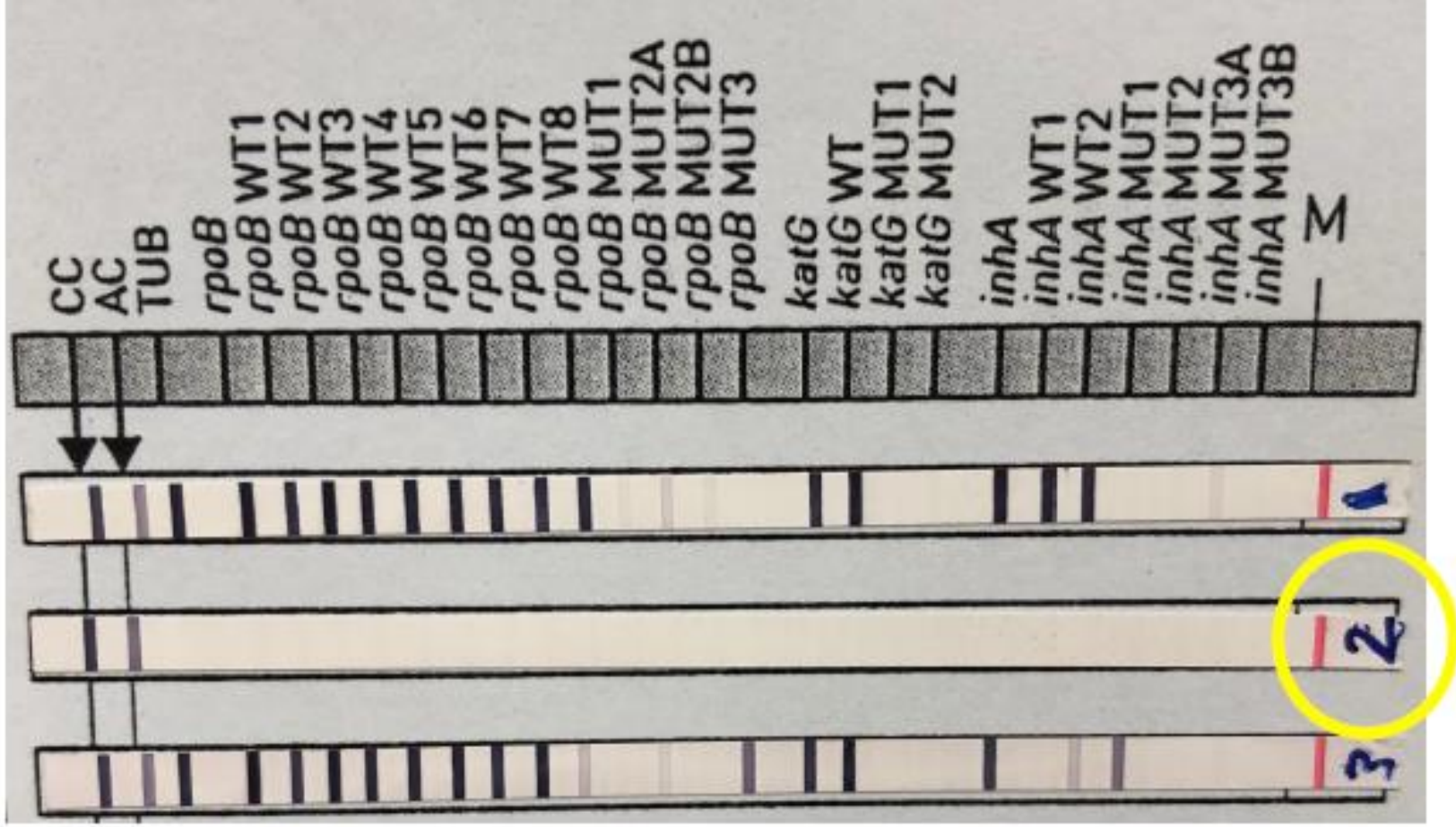

#1: CC, AC and TUB bands all present: This is a **valid** positive MTBC result.

#2: CC, AC bands are present, but TUB band is absent: This is a **valid negative** MTBC result.

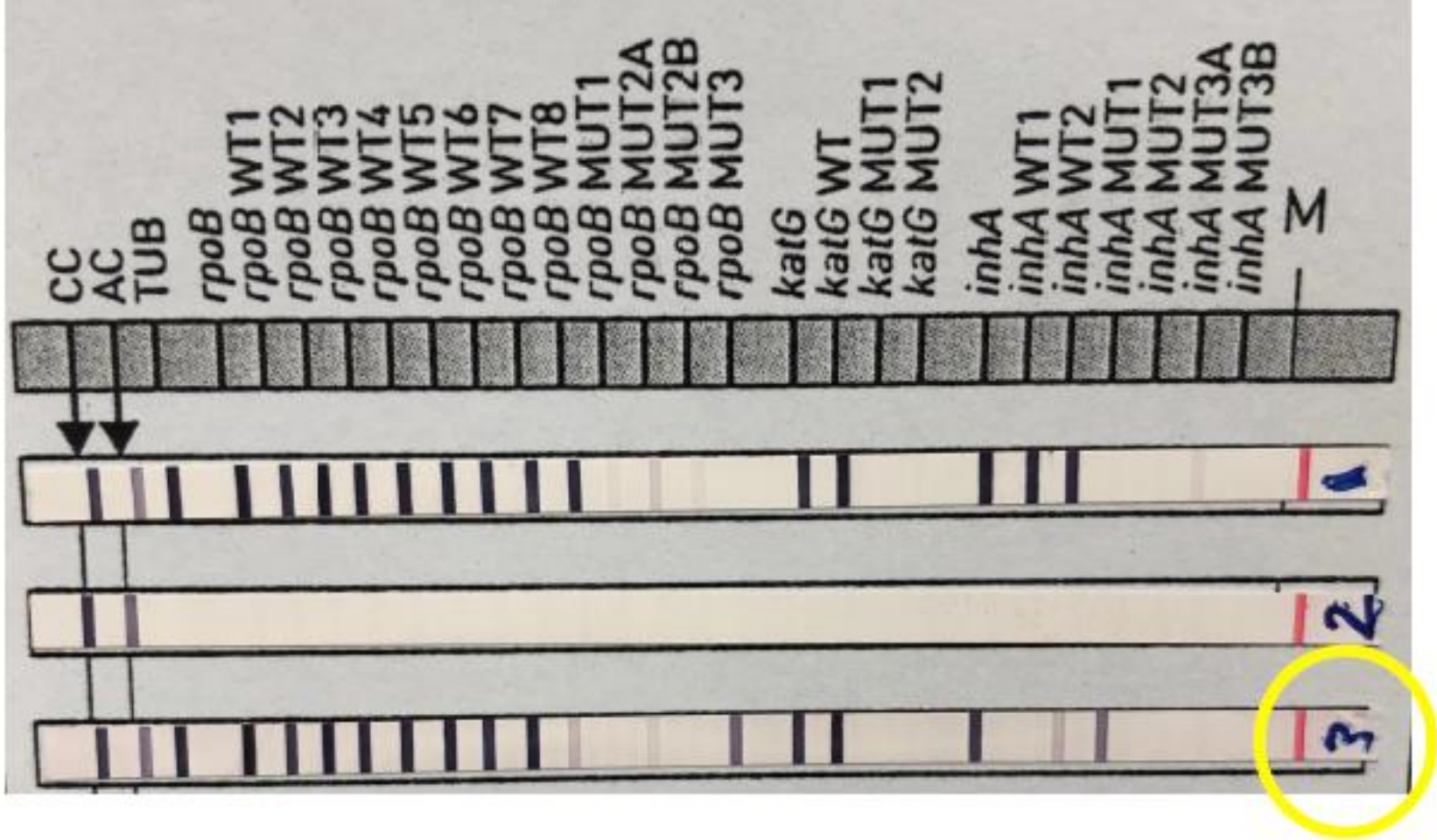
#3: CC, AC and TUB bands all present: This is a **valid** positive MTBC result.




#1: All 3 drug control loci (rpoB/katG/inhA) have bands present, so the mutation data is **valid**.
ing the WT and MUT bands to the AC band, all WT bands present, and there are no MUT bands
darker than the AC band. Therefore this is an **MTBC strain showing no mutations for INH or RIF**.




#2: There are only CC and AC bands present. Since this is a valid negative result, there are **no for susceptibility.**




3: All 3 drug control loci (rpoB/katG/inhA) have bands present, so the mutation data is **valid**.

WT bands are present, and there is 1 MUT band (MUT3) that is darker than the AC band

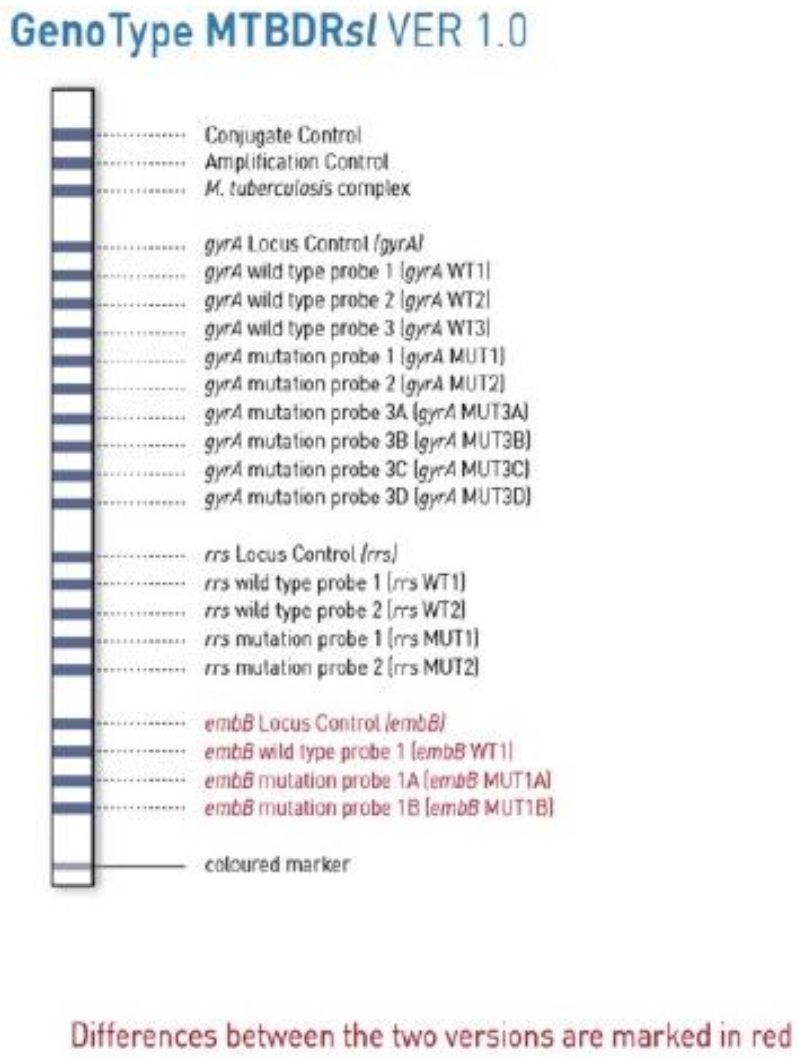
**y: This indicates a RIF mutation that may correlate with resistance**

ne WT band is NOT present for inhA WT1, but faintly present for WT2. In addition, there is a MUT band present (MUT1).

# Interpreting Line Probe Assay Strips: MTBDRsl

- Interpret the same way as the MTBDRplus assay
  - CC/AC/TUB control present?
  - Drug control bands present?
  - Review WT and MUT bands

**Make sure to interpret based on the correct test version!**



## le #1:

AC and TUB bands present: MTB complex (+)

g control bands present

bands present for all drugs

MUT bands present

**retation: MTB complex (+), no mutations found**

## le 2:

AC bands present, TUB bands absent: MTB complex (-)

g control bands absent

bands absent for all drugs

MUT bands present

**retation: MTB complex (-)**

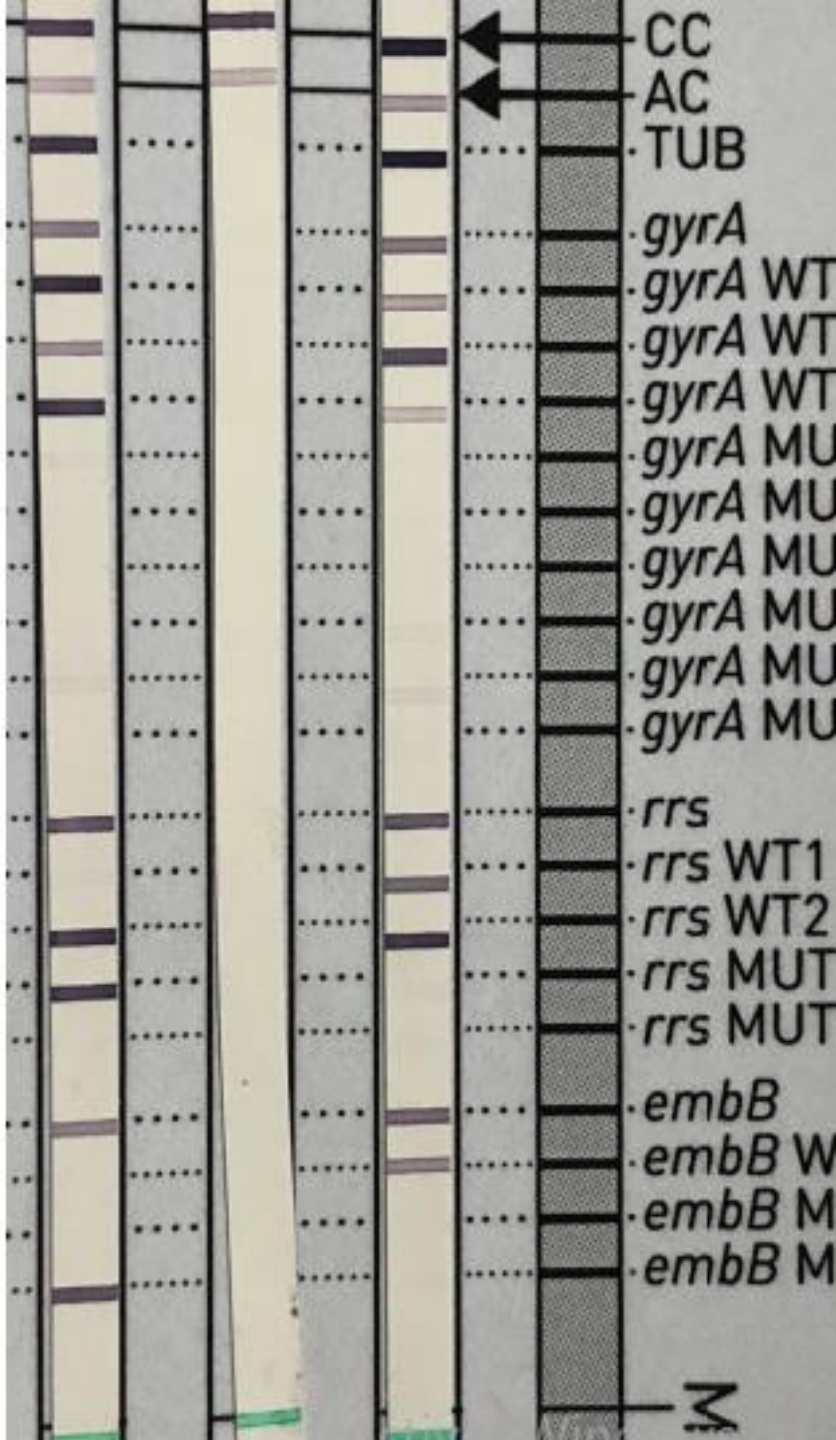
## le 3:

AC and TUB bands present: MTB complex (+)

g control bands present

bands present for gyrA and embB, missing WT1 for rrs

T band present for rrs (MUT1) and embB (MUT1B)



# GenoType MTBDRplus version 2.0

## Attributes and advantages

- Highly sensitive and specific (98.9%, 100%)
- Detects both Rifampicin and Isoniazid
- Meant for low and high throughput labs
- Short Turn around time of 5hrs
- Affordable – ~$10 per test

## Disadvantages

- Cannot be used as point of care test
- Requires biosafety facilities

# Benefits of the assay (1)

- Highly Sensitive and specific: Test can be performed from +ve and –ve sputum smear samples
- Efficient diagnosis: Patients previously tested and are MDR-TB positive don't need to give out another sputum sample
- Rapid XDR-TB results generated within 4 hours

# Benefits of the assay (2)

- Improved version contains extra genes (gyrB gene) in fluoroquinolones. This reduces negative/positives cases
- Enables early patient isolation and therapy initiation preventing spread of the strain
- Laboratory procedure remains the same

# Thank you for your attention