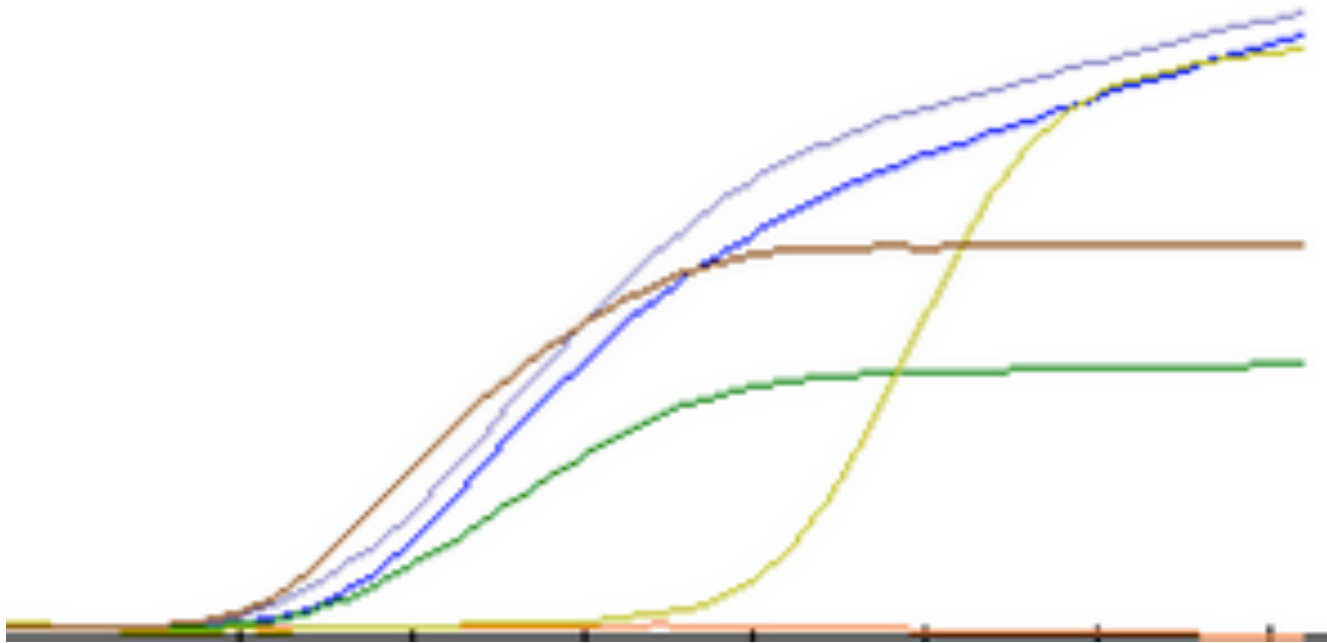


## Xpert® MTB/RIF (Cepheid)

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Here is presented the current MTB/RIF test.

The World Health Organization (WHO) today recommended the use in all settings of a next-generation Xpert® MTB/RIF assay (called Xpert® MTB/RIF Ultra) as a replacement for the current Xpert® MTB/RIF cartridge (24 March 2017) that will be presented during the talks.

## Principle

The Xpert MTB/RIF test for use with the Cepheid GeneXpert® System is a semi-quantitative nested real-time PCR *in vitro* diagnostic test for: 1) the detection of *Mycobacterium tuberculosis* complex (MTB complex) DNA in sputum samples or concentrated sediments prepared from induced or expectorated sputa that are either acid-fast bacilli (AFB) smear positive or negative; 2) the detection of rifampin-resistance associated mutations of the *rpoB* gene in samples from patients at risk for rifampin resistance.



## 1. Material

### 1. Reagents Material

- GeneXpert Dx System equipped with Gx Dx software version 4.3 or higher, GeneXpert instrument, computer, barcode reader, and Operator Manual
- Optional Printer
- The Xpert MTB/RIF kit (GXMTB/RIF-10 or GXMTB/RIF-50) contains sufficient reagents to process 10/50 patient or quality-control specimens:
  - Sample Reagent (Sodium Hydroxide and Isopropanol, 8 mL per cartridge which is clear, ranging from colorless to golden yellow),
  - Sterile disposable transfer pipettes with a single mark representing the minimum volume of decontaminated sample to transfer to the Xpert MTB/RIF cartridge.



- Sterile screw-capped specimen collection containers
- Disposable Gloves
- Labels and/or indelible labeling marker
- Sterile pipettes (2 mL) for sample processing

### 2. Storage and Handling

- Store the Xpert MTB/RIF cartridges and reagents at 2–28 °C.
- Do not use reagents or cartridges that have passed the expiration date.
- Do not open a cartridge until you are ready to perform testing.
- Use the cartridge within 4 hours after opening the cartridge lid.

- The cartridge is stable up to 7 days after opening the package (Kit of 50).

### 3. Warnings and Precautions

- The performance of Xpert MTB/RIF for the detection of MTB complex has not been demonstrated from non-respiratory specimens such as blood, CSF, stool or urine. The performance of the Xpert MTB/RIF test has not been evaluated with specimens processed by methods other than those described in this package insert.
- Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents. Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Do not open the Xpert MTB/RIF cartridge lid except when adding sample.
- Do not use a cartridge that has been dropped or shaken after you have added the treated sample.
- Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert MTB/RIF cartridge is used to process one test. Do not reuse spent cartridges.
- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents.
- Consult your institution's environmental waste personnel on proper disposal of used cartridges and unused reagents.

## 2. Procedure

### 1. Specimen Collection and Transport

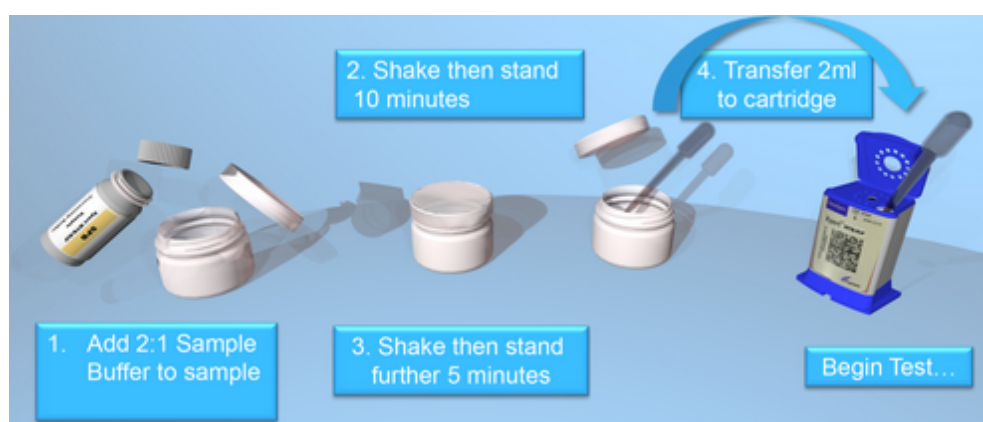
- Collect a minimum of 1 mL of sputum per specimen. Subject must be seated or standing.
- Specimens should be held at 2–8 °C prior to processing whenever possible. However, if necessary the specimens can be stored at a maximum of 35 °C for ≤3 days and then at 2-8 °C for an additional 7 days.
  - 1) Rinse the patient's mouth twice with water.
  - 2) Unscrew the lid on the sputum collection container.
  - 3) Have the patient inhale deeply, cough vigorously, and expectorate the material into the sterile screw-capped specimen collection container. Avoid spills or soiling the outside of the container.
  - 4) Secure the lid on the collection device.
  - 5) Specimens should be held at 2–8 °C whenever possible including during transport to the laboratory.

## 2. Expecterated Sputum Samples

- Do not accept specimens with obvious food particles or other solid particulates.
- Process only as many samples at one time as there are modules available to run the test on the GeneXpert Dx System.
- Guidelines for handling TB should be closely followed:
  - 1) Label each Xpert MTB/RIF cartridge with the sample ID. (Write on the sides of the cartridge or affix ID label.) Note: Do not put the label on the lid of the cartridge or obstruct the existing 2D barcode on the cartridge.
  - 2) Leave specimen in leak-proof sputum collection container.
  - 3) For each of the samples:
    - a. Unscrew lid of sputum collection container
    - b. Add Sample Reagent 2:1 (v/v) to sample,
    - c. Replace the lid, and shake vigorously 10 - 20 times.

Note: One back-and-forth movement is a single shake.

- 4) Incubate for 15 minutes at room temperature. At one point between 5 and 10 minutes of the incubation again shake the specimen vigorously 10 – 20 times. Samples should be liquefied with no visible clumps of sputum. Particulate matter may exist that is not part of the sample.



## 3. Sputum Sediments

- Do not accept specimens with obvious food particles or other solid particulates.
- Process only as many samples at one time as there are modules available to run the test on the GeneXpert Dx System.
- Sputum sediments prepared according to the method of Kent and Kubica and re-suspended in 67mM Phosphate/H<sub>2</sub>O buffer can be tested using Xpert MTB/RIF. Once the re-suspension is prepared for standard laboratory smear or culture tests, ensure at least 0.5 mL of re-suspended sediment is available to run Xpert MTB/RIF.

- 1) Label each Xpert MTB/RIF cartridge with the sample ID. (Write on the sides of the cartridge or affix ID label.) Note: Do not put the label on the lid of the cartridge or obstruct the existing 2D barcode on the cartridge.
- 2) Transfer at least 0.5 mL of the total re-suspension pellet to a conical, screw-capped tube for the Xpert MTB/RIF using a sterile transfer pipette. Alternatively, the entire sample may be processed in the original tube.
- 3) Store re-suspended sediments at 2–8 °C if they are not immediately processed for Xpert MTB/RIF. Do not store for more than 12 hours.
- 4) Add 1.5 mL of Xpert MTB/RIF Sample Reagent (SR) to 0.5 mL of re-suspended sediment sample using a sterile transfer pipette.



- 5) Shake vigorously 10 – 20 times. Note: One back-and-forth movement is a single shake.



- 6) Incubate the specimen for 15 minutes at room temperature. At one point between 5 and 10 minutes of the incubation, again shake the specimen vigorously 10 – 20 times. Samples should be liquefied with no visible clumps of sputum. Particulate matter may exist that is not part of the sample.

#### 4. Preparing the Cartridge

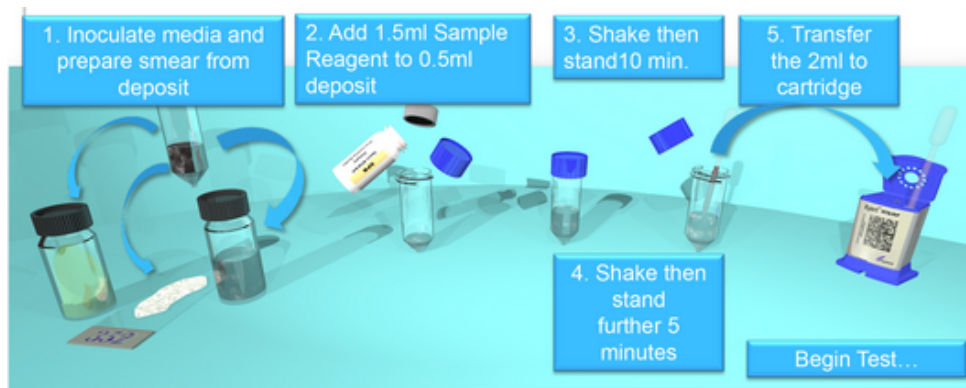
Important: Start the test within 30 minutes of adding the sample to the cartridge.

- 1) Using the sterile transfer pipette provided, aspirate the liquefied sample into the transfer pipette until the meniscus is above the minimum mark. Do not process the sample further if there is insufficient volume.
- 2) Open the cartridge lid. Transfer sample into the open port of the Xpert MTB/RIF cartridge. See picture below. Dispense slowly to minimize the risk of aerosol formation.



- 3) Close the cartridge lid. Make sure the lid snaps firmly into place. Remaining liquefied sample may be kept for up to 12 hours at 2 – 8 °C should repeating the test be required.

Important: Be sure to load the cartridge into the GeneXpert Dx instrument and start the test within 30 minutes of preparing the cartridge.



## 5. Starting the Test

Important: Before you start the test, ensure that the system is equipped with the GX Dx software version 4.3 or higher, and the Xpert MTB/RIF assay definition file is imported into the software.

This section lists the basic steps of running the test.

For detailed instructions, see the GeneXpert Dx System Operator Manual.

- 1) Turn on the computer, and then turn on the GeneXpert Dx instrument.
- 2) On the Windows® desktop, double-click the GeneXpert Dx shortcut icon.
- 3) Log on to the GeneXpert Dx System software using your user name and password.
- 4) In the GeneXpert Dx System window, click Create Test.
- 5) Scan or type in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is associated with the test results and is shown in the View Results window.
- 6) Scan or type in the Sample ID. If typing the Sample ID, make sure the Sample ID is typed correctly. The Sample ID is associated with the test results and is shown in the View Results window and all reports.
- 7) Scan the barcode on the Xpert MTB/RIF cartridge. The Create Test window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
- 8) Click Start Test. In the dialog box that appears, type your password if requested.
- 9) Open the instrument module door with the blinking green light and load the cartridge.

- 10) Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
- 11) Wait until the system releases the door lock at the end of the run, then open the module door and remove the cartridge.
- 12) Dispose of used cartridges in the appropriate specimen waste containers according to your institution's standard practices.

### 3. Interpretation of Results

#### 1. Quality Control

Each test includes a Sample Processing Control (SPC) and probe check (PCC).

Sample Processing Control (SPC) ensures the sample was correctly processed. The SPC contains non-infectious spores in the form of a dry spore cake that is included in each cartridge to verify adequate processing of MTB. The SPC verifies that lysis of MTB has occurred if the organisms are present and verifies that specimen processing is adequate. Additionally, this control detects specimen-associated inhibition of the real-time PCR assay. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria. The test result will be “Invalid” if the SPC is not detected in a negative test.

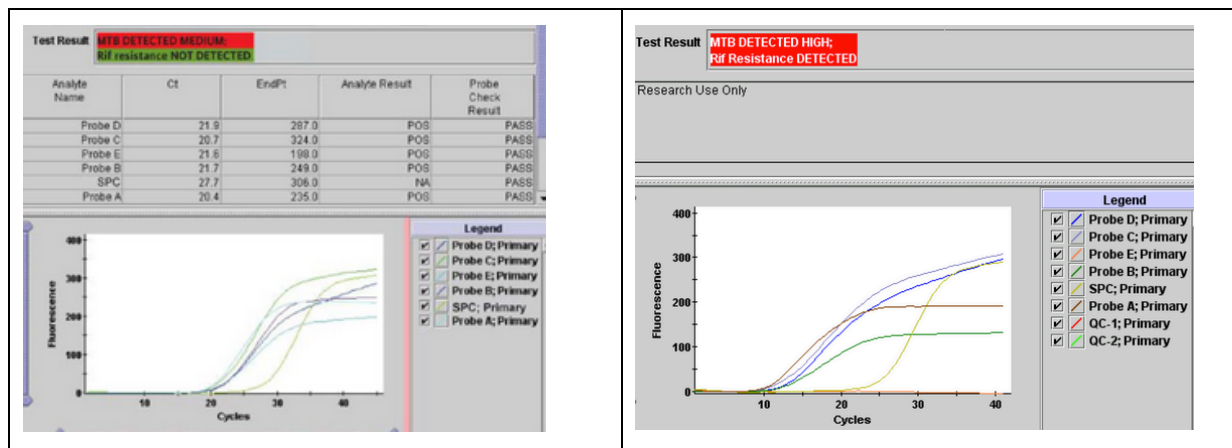
Probe Check Control (PCC): Before the start of the PCR reaction, the GeneXpert Dx System measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. Probe Check passes if it meets the assigned acceptance criteria.

#### 2. Results

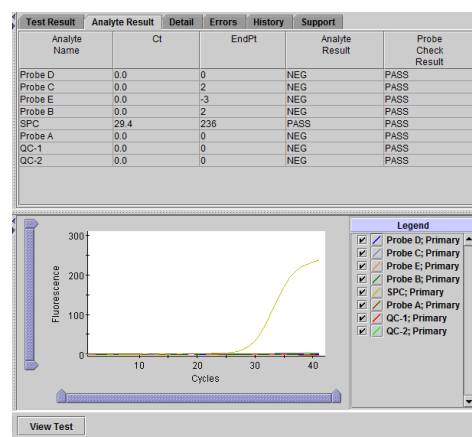
The results are interpreted by the GeneXpert DX System from measured fluorescent signals and embedded calculation algorithms and will be displayed in the “View Results” window. Lower Ct values represent a higher starting concentration of DNA template; higher Ct values represent a lower concentration of DNA template.

Ct range : High <16, Medium 16-22, Low 22-28, Very Low >28

## MTB Detected: MTB target DNA is detected.



- MTB Detected—The MTB result will be displayed as High, Medium, Low or Very Low depending on the Ct value of the MTB target present in the sample.
- Rif Resistance DETECTED, Rif Resistance NOT DETECTED, or Rif Resistance INDETERMINATE will be displayed only in MTB DETECTED results and will be on a separate line from the MTB DETECTED result.
  - ✓ Rif Resistance DETECTED; a mutation in the *rpoB* gene has been detected that falls within the valid delta Ct setting.
  - ✓ Rif Resistance INDETERMINATE; the MTB concentration was very low and resistance could not be determined.
  - ✓ Rif Resistance NOT DETECTED; no mutation in the *rpoB* gene has been detected.
- SPC— NA (not applicable); SPC signal is not required since MTB amplification may complete with this control.
- Probe Check—PASS; all probe check results pass.



## MTB Not Detected: MTB target DNA is not detected, SPC meets acceptance criteria.

- MTB NOT DETECTED—MTB target DNA is not detected.
- SPC— Pass; SPC has a Ct valid range and endpoint above the endpoint minimum setting.
- Probe Check—PASS; all probe check results pass.

## INVALID

Presence or absence of MTB cannot be determined, repeat test with extra specimen. SPC does not meet acceptance criteria, the sample was not properly processed, or PCR is inhibited.

- MTB INVALID—Presence or absence of MTB DNA cannot be determined.
- SPC—FAIL; MTB target result is negative and the SPC Ct is not within valid range.
- Probe Check—PASS; all probe check results pass.

## ERROR

- MTB—NO RESULT
- SPC—NO RESULT
- Probe Check—FAIL\*; one or more of the probe check results fail. \*If the probe check passed, the error is caused by a system component failure.

## NO RESULT

- MTB—NO RESULT
- SPC—NO RESULT
- Probe Check—NA (not applicable)

## 4. Reasons to Repeat the Assay

Repeat the test using a new cartridge or initiate alternate procedures if one of the following test results occurs:

- An INVALID result indicates that the SPC failed. The sample was not properly processed or PCR was inhibited.
- An ERROR result indicates that the Probe Check control failed and the assay was aborted possibly due to the reaction tube being filled improperly, a reagent probe integrity problem was detected, or because the maximum pressure limits were exceeded or there was a GeneXpert module failure.
- A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

## 5. Limitations

The performance of the Xpert MTB/RIF was validated using the procedures provided in this package insert. Modifications to these procedures may alter the performance of the test. Results from the Xpert MTB/RIF should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

Because the detection of MTB is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage. Erroneous test results might occur from improper specimen

collection, failure to follow the recommended sample collection procedure, handling or storage, technical error, sample mix-up, or an insufficient concentration of starting material. Careful compliance to the instructions in this insert is necessary to avoid erroneous results.

A positive test result does not necessarily indicate the presence of viable organisms. It is however, presumptive for the presence of MTB and Rifampicin resistance.

Test results might be affected by prior or concurrent antibiotic therapy. Therefore, therapeutic success or failure cannot be assessed using this test because DNA might persist following antimicrobial therapy.

Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown MDR-MTB or rifampicin resistant strains resulting in a false negative result.

## 6. Maintenance

- Preventative maintenance tasks:
  - Performed on regular basis by end-users: daily, weekly, monthly, yearly
  - Ensures good performance of the system
  - Helps to avoid GeneXpert malfunctions
- Required tool/products:
  - 1:10 dilution of household chlorine bleach\* (used within 1 day of preparation)
  - 70% ethanol solution
  - Lint-free wipes / non-cotton swabs
  - Brushes (when provided with system/available upon request)
  - Disposable gloves
  - Clean water
  - Eye protection
  - Replacement fan filters (available upon request)

**Daily Maintenance:** Clean work area / Remove and discard used cartridges/  
Keep the doors upright

**Weekly Maintenance:** Power down the GeneXpert computer/ Power down the GeneXpert Instrument/ Turn on the GeneXpert /Turn on the on GeneXpert computer

**Monthly Maintenance:**

Clean instrument surfaces/ Clean cartridge bays/Clean plunger rods/Clean module PCR tube slots/Clean fan filter/Archive and purge (delete) runs

**Yearly Maintenance:**

Thermal AND Optical Calibration of the module(s): Verification / Adjustment for system to work and perform within a set of specifications with Xpert Check kit

Contact Hotline to learn more about the maintenance process:

***MTB/RIF on Tuesday October 23 2018***

## 7. Bibliographic references

- Boehme, CC, P Nabeta, D Hillemann, et al. 2010. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 363:1005-1015.
- Boehme, CC, MP Nicol, P Nabeta, et al. 2011. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet 377:1495-1505.
- Hillemann, D, Rüsç-Gerdes S, Boehme C, Richter E. 2011. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. J Clin Microbiol 49:1202-1205.
- Trebucq, A, DA Enarson, CY Chiang, et al. 2011. Xpert(R) MTB/RIF for national tuberculosis programmes in low-income countries: when, where and how? Int J Tuberc Lung Dis 15:1567-1572.
- WHO. 2011. Rapid Implementation of the Xpert MTB/RIF diagnostic test  
Technical and Practical considerations

### Training module

The GLI has developed a set of training modules on Xpert MTB/RIF, which are now available for download at:

[http://www.stoptb.org/wg/gli/TrainingPackage\\_XPERT\\_MTB\\_RIF.asp](http://www.stoptb.org/wg/gli/TrainingPackage_XPERT_MTB_RIF.asp) (may 2014)

These modules are in Powerpoint format, allowing for country customization, and include the following topics:

Overview of TB and TB diagnostics; Biosafety; Collection and transportation of specimens; Supplies management; Installation; GeneXpert technology and Xpert MTB/RIF procedures; Results interpretation and database management; Recording and reporting; Troubleshooting; Maintenance; Clinical guide to Xpert MTB/RIF

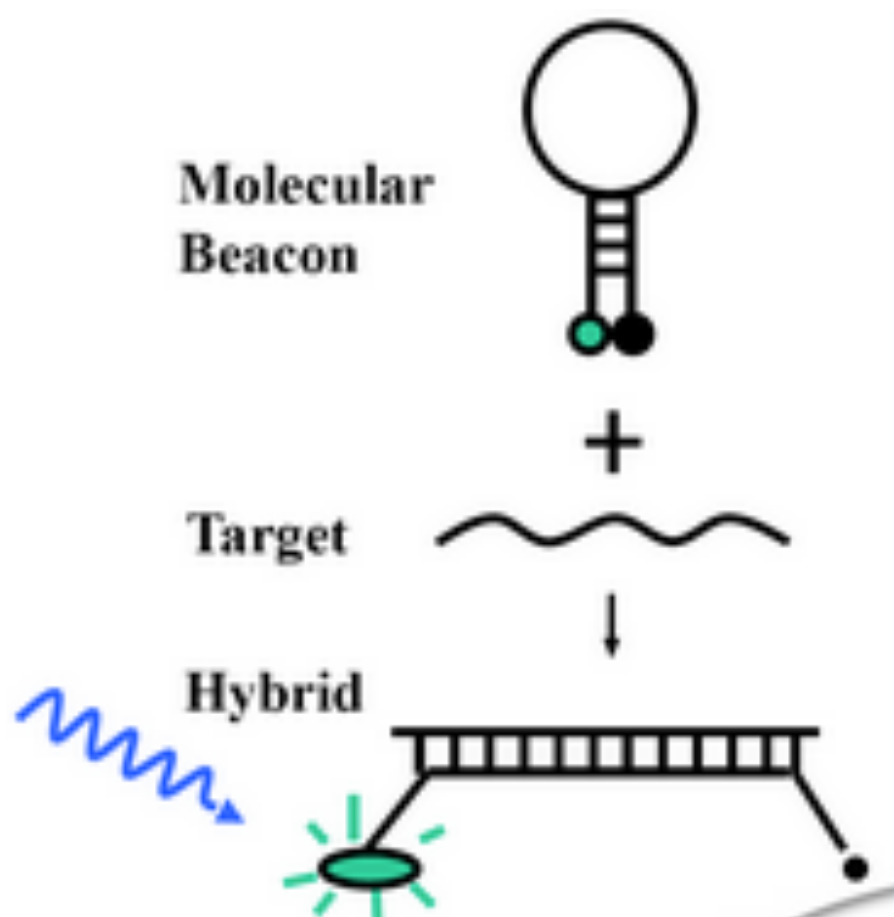
The modules have combined and updated existing materials from KNCV, FIND and Cepheid. A module on quality assurance is forthcoming, as well as a facilitator guide, customization guide and participant manual.

The modules are a TB CARE I product made possible with USAID support, and have been developed by a core team of GLI partners:

- KNCV (Valentina Anisimova, Sanne van Kampen)
- FIND (Heidi Albert)
- CDC (Heather Alexander, Tom Shinnick)
- USAID (Amy Piatek)
- WHO (Diego Zallocco, Chris Gilpin, Wayne van Gemert)

## SUPPLEMENT 1

### Principle of Molecular Beacon reaction



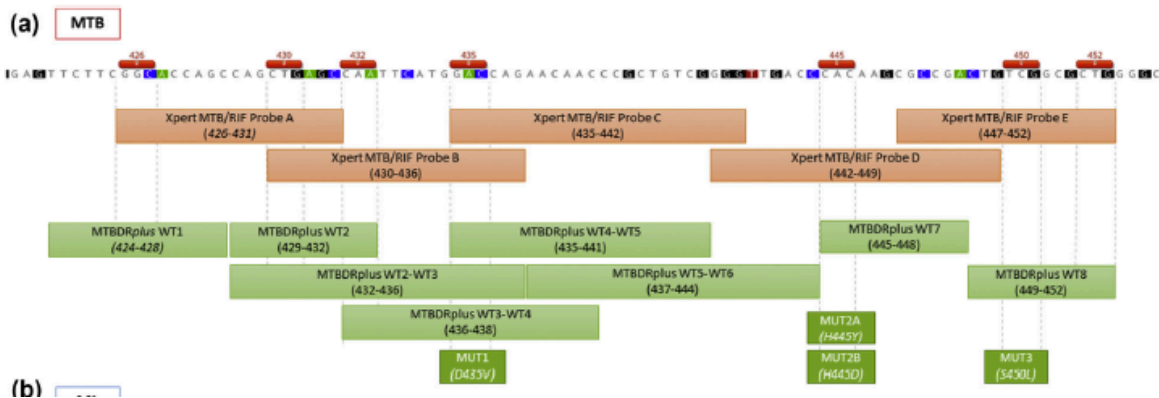
# SUPPLEMENT 2

## *rpoB* gene 81 bp RIF-resistance determining region and Xpert MTB/RIF and MTBDR*plus* probes



## *rpoB* gene 81 bp RIF-resistance determining region and Xpert MTB/RIF probes

*E. Andre et al / Clinical Microbiology and Infection 23 (2017) 167–172*



## *rpoB* gene 81 bp RIF-resistance determining region and Xpert MTB/RIF and MTBDR*plus* probes