Mycobacteria Product Series: GenoType Test Systems

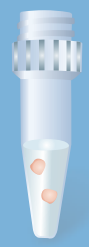
(A) DNA Extraction Area, BSL 2/BSL 3 Laboratory

- Put on new gloves and lab coat.
- Decontaminate work area with freshly diluted 1.5% sodium hypochlorite solution.
- If Internal Control DNA (IC) is provided with the **GenoType** kit (**GenoType Mycobacterium CM VER 2.0/AS, GenoType CM*direct***), then prepare A-LYS/IC mix (A-LYS provided with **GenoLyse®** kit) for all samples and negative control first. Use 100 µl Lysis Buffer (A-LYS) and 2 µl IC per sample.
- If a negative control shall be included, use 100 µl A-LYS or A-LYS/IC mix and continue with **GenoLyse®** procedure, step 3.

(A) DNA Extraction

Starting material

GenoType MTBDR*plus/sl* VER 2.0
GenoType CM*direct*



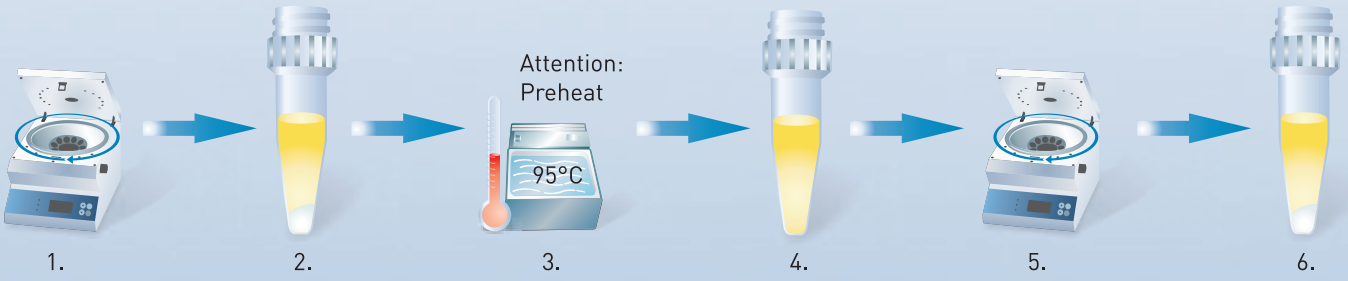
Patient specimen: Use 500 µl decontaminated patient specimen.

GenoType MTBDR*plus/sl* VER 2.0, GenoType Mycobacterium CM VER 2.0/AS, GenoType MTBC, GenoType NTM-DR

Bacteria grown in liquid medium: Use 1 ml of culture.

Bacteria grown on solid medium: Collect with inoculation loop, suspend in 100 µl A-LYS or A-LYS/IC mix, vortex and continue with **GenoLyse®** procedure, step 3.

DNA extraction with GenoLyse®



1. Centrifuge for 15 min at 10,000 x g in a centrifuge with aerosol-tight rotor, carefully remove supernatant.
2. Add 100 µl A-LYS or A-LYS/IC mix to pellet and vortex.
3. Incubate for 5 min at 95°C in a water bath, briefly spin down.
4. Add 100 µl Neutralization Buffer (A-NB) and vortex for 5 sec.
5. Centrifuge for 5 min at full speed.
6. = DNA solution
   For long-term storage, transfer supernatant to a new tube and store at -20°C.

- Decontaminate pipettes, tip boxes, racks and work area with freshly diluted 1.5% sodium hypochlorite solution.

Please make sure that the procedure described in this flow chart corresponds to the instruction for use (IFU) of the kit at hand.
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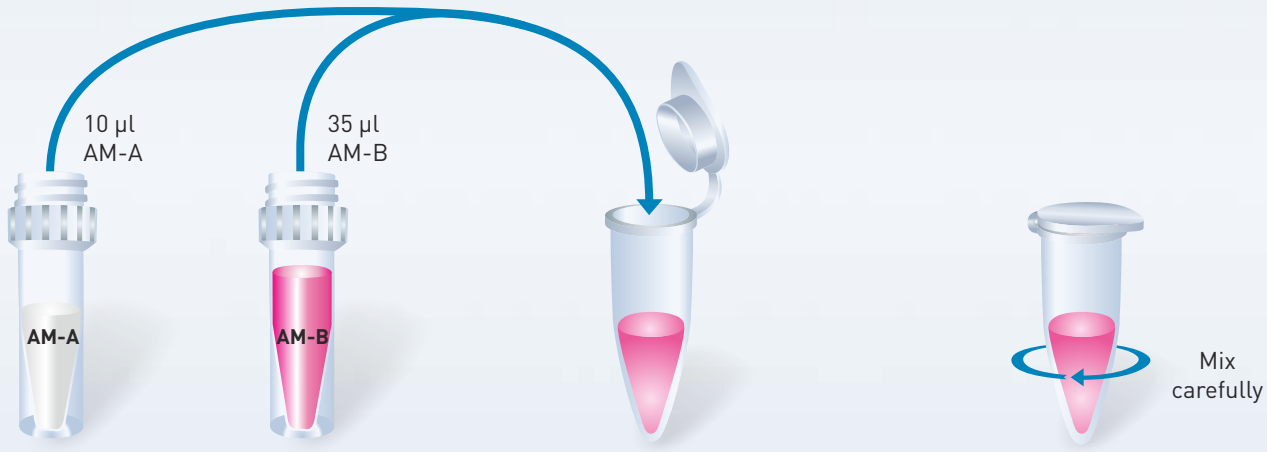
Mycobacteria Product Series: GenoType Test Systems

(B) Pre-PCR Area, BSL 1 Laboratory

- Put on new gloves and lab coat.
- Decontaminate work area with freshly diluted 1.5% sodium hypochlorite solution.
- Avoid handling and pipetting above open vials, close lids after each pipetting step.
- Whenever opening new AM-A or AM-B, aliquot and store at -20°C.

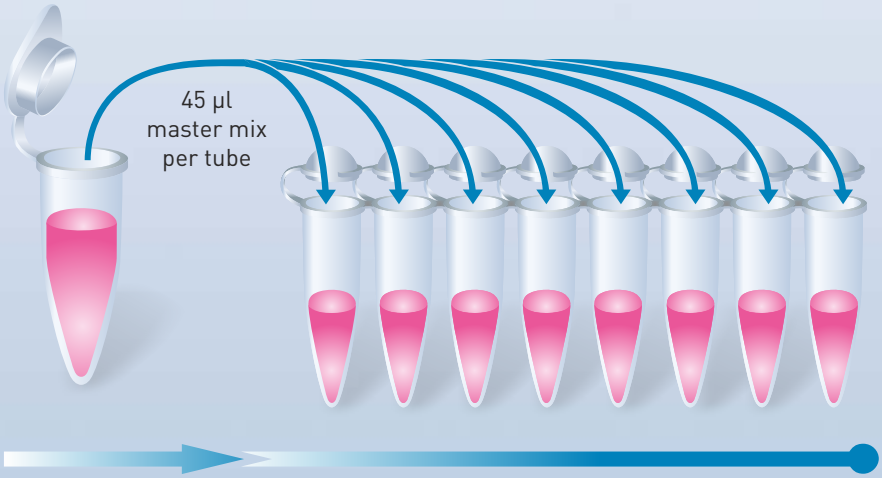
(B) PCR Master Mix

Prepare master mix containing AM-A and AM-B (per sample)



- Master mix needs to be prepared freshly each time.
- Do not vortex master mix.
- Determine the number of samples to be analysed plus controls and prepare master mix accordingly.

Aliquot master mix into PCR tubes



- Decontaminate pipettes, tip boxes, racks and work area with freshly diluted 1.5% sodium hypochlorite solution.

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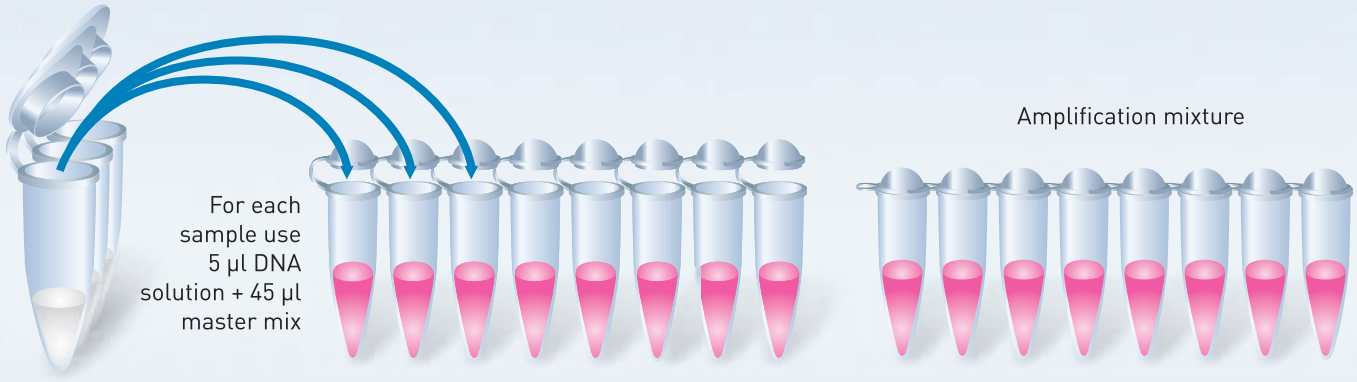
Mycobacteria Product Series: GenoType Test Systems

(C) PCR Area, BSL 1 Laboratory

- Put on new gloves and lab coat.
- Decontaminate work area with freshly diluted 1.5% sodium hypochlorite solution.

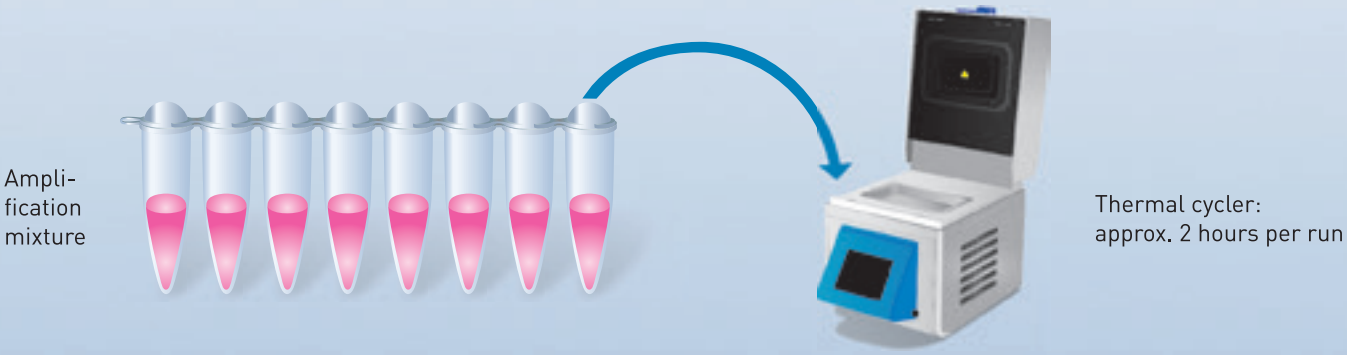
(C) PCR Area

Add DNA solution to each master mix aliquot



- Volumes described are applicable for DNA extracted with **GenoLyse®**.
- For negative control use 5 µl water or 5 µl negative control isolate instead of DNA solution.
- If a positive control shall be included, pipette 5 µl C+ (**GenoType Mycobacterium CM VER 2.0/AS**, **GenoType CM*direct***) to one aliquot. Open and pipette C+ last and close vial immediately afterwards.

Place PCR tubes into thermal cycler



- When using a thermal cycler with the respective pre-installation, select specific protocol for patient specimens or cultivated samples.

- Decontaminate pipettes, tip boxes, racks and work area with freshly diluted 1.5% sodium hypochlorite solution.

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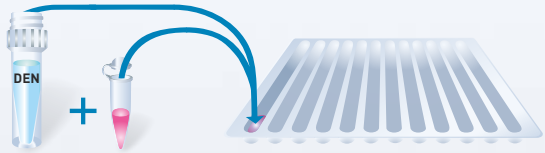
Mycobacteria Product Series: GenoType Test Systems

(D-E) Hybridization and Detection Area, BSL 1 Laboratory

- Put on new gloves and lab coat.
- Decontaminate work area with freshly diluted 1.5% sodium hypochlorite solution.
- Pre-warm RIN and distilled water to room temperature (RT) and HYB and STR to 45°C.
- Freshly dilute CON-C 1:100 with CON-D and SUB-C 1:100 with SUB-D in the amounts needed.

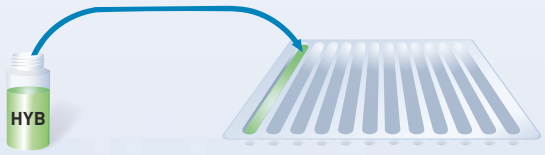
Use **TwinCubator** with program for the hybridization and detection procedure

(D) Hybridization



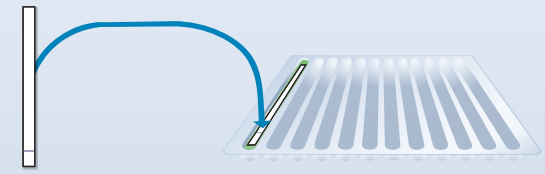
20 µl DEN + 20 µl Amplicon

Mix and incubate for 5 min at RT

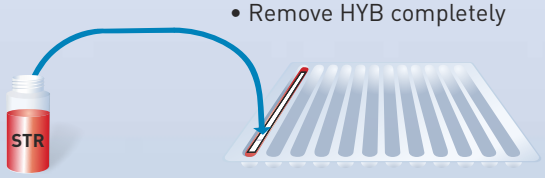


1 ml HYB

Add and mix carefully

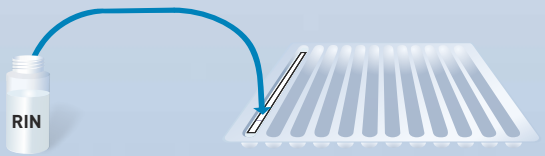


- Place **DNA•STRIP** in well
- Incubate for 30 min at 45°C
- Remove HYB completely



1 ml STR

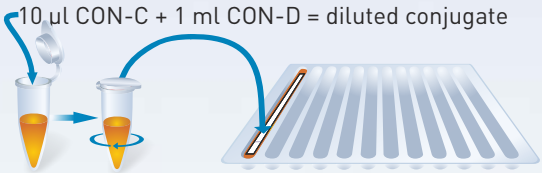
- Incubate for 15 min at 45°C
- Remove STR completely



1 ml RIN

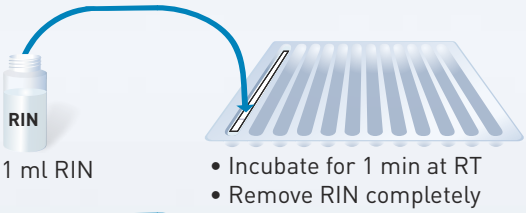
- Incubate for 1 min at RT
- Remove RIN completely

(E) Detection



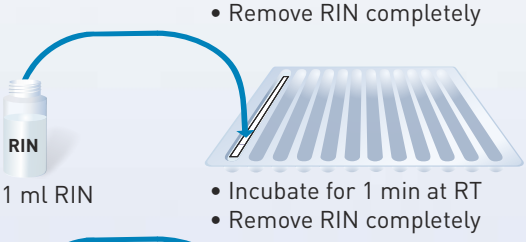
10 µl CON-C + 1 ml CON-D = diluted conjugate

- Incubate for 30 min at RT
- Remove conjugate completely



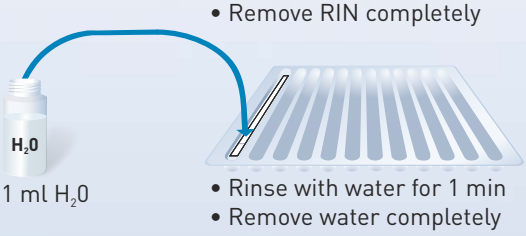
1 ml RIN

- Incubate for 1 min at RT
- Remove RIN completely



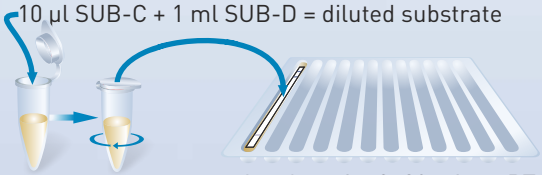
1 ml RIN

- Incubate for 1 min at RT
- Remove RIN completely



1 ml H_2O

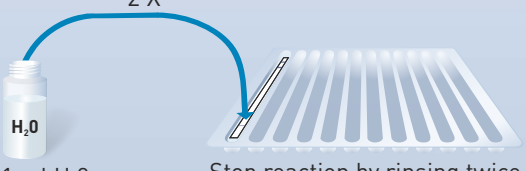
- Rinse with water for 1 min
- Remove water completely



10 µl SUB-C + 1 ml SUB-D = diluted substrate

- Incubate for 3–20 min at RT
- Remove substrate completely

2 X



1 ml H_2O

Stop reaction by rinsing twice with water

Remove **DNA•STRIP** from tray and dry it on absorbent paper, evaluate results using template

- Decontaminate pipettes, tip boxes, racks and work area with freshly diluted 1.5% sodium hypochlorite solution.

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