GenoType® MTBDRsl

Identification of the M. tuberculosis complex and its resistance to fluoroquinolones, aminoglycosides/cyclic peptides and ethambutol

Objective

Detection of XDR-TB by Detection of XDR-TB in patients previously diagnosed with an MDR-TB.

DNA Extraction

- From bacteria grown on solid medium: collect bacteria with an inoculation loop and suspend in 200 µl TE buffer
- From bacteria grown on liquid media: spin 1,5 ml MGIT and suspend the pellet in 200 µl TE buffer
- From smear-positive clinical specimen: spin 500 µl decontaminated sample and suspend the pellet in 200 µl TE buffer

Material
- 200 µl TE buffer aliquots [TE 20 µl EDTA 0,5M + 100 µl Tris HCl 1M + water to 10 ml]
- 20µl microbeads
- 1 µl sterile loops
- Vortex (PSM hood)
- Store – 20°C

DNA Extraction
- Bacteria grown in solid or liquid medium or smear-positive clinical specimens
- Into 200 µl TE buffer with 20µl microbeads
- 95°C, 20 min
- Vortex
- Spin down 1 min at full speed
- – 20°C (at least 30 min)
- Directly use 5 µl of the supernatant for PCR

Amplification

Prepare the amplification mix (50 µl) in a DNA-free room. The DNA sample should be added in a separated area.

Per tube, mix:
- 35 µl PNM
- 5 µl 10X polymerase incubation buffer
- 2 µl MgCl2 (25mM) [Final 2.5 mM]
- 1-2 U Taq DNA polymerase
- water to 45 µl
- Mix well
- Aliquot 45 µl of master mix in each of the prepared PCR tubes
- Add 5 µl DNA solution

Optional agarose gel (2%) (60 – 120 bp)
Hybridization

Preparation
Prewarm shaking water bath/TwinCubator® to 45 °C; the maximum tolerated deviation from the target temperature is +/-1 °C. Prewarm solutions HYB and STR to 37-45 °C before use. The reagents must be free from precipitates (note, however, that solution CON-D is opaque). Mix if necessary. Warm the remaining reagents with the exception of CON-C and SUB-C to room temperature. Using a suitable tube, dilute Conjugate Concentrate (CON-C, orange) and Substrate Concentrate (SUB-C, yellow) 1:100 with the respective buffer (CON-C with CON-D, SUB-C with SUB-D) in the amounts needed. Mix well and bring to room temperature. For each strip, add 10 µl concentrate to 1 ml of the respective buffer. Dilute CON-C before each use. Diluted SUB-C is stable for 4 weeks if stored at room temperature and protected from light.

1. Dispense 20 µl of Denaturation Solution (DEN, blue) in a corner of each of the wells used.
2. Add to the solution 20 µl of amplified sample, pipette up and down to mix well and incubate at room temperature for 5 min. Meanwhile, take strips out of the tube using tweezers and mark them with a pencil underneath the colored marker. Always wear gloves when handling strips.
3. Carefully add to each well 1 ml of prewarmed Hybridization Buffer (HYB, green). Gently shake the tray until the solution has a homogenous color. Take care not to spill solution into the neighboring wells.
4. Place a strip in each well. The strips must be completely covered by the solution and the coated side (identifiable by the colored marker near the lower end) must face upward. Using tweezers, turn over strips which might have turned when immersed in the solution. Carefully clean tweezers after each use to avoid contamination. This also applies to all following steps.
5. Place tray in shaking water bath/TwinCubator® and incubate for 30 min at 45 °C. Adjust the shaking frequency of the water bath to achieve a constant and thorough mixing of the solution. To allow adequate heat transfer, the tray must be dipped into the water to at least 1/3 of its height.
6. Completely aspirate Hybridization Buffer. For example, use a Pasteur pipette connected to a vacuum pump.
7. Add 1 ml of Stringent Wash Solution (STR, red) to each strip and incubate for 15 min at 45 °C in shaking water bath/TwinCubator®.
8. Work at room temperature from this step forward. Completely remove Stringent Wash Solution. Pour out Wash Solution in a waste container and remove all remaining fluid by turning the tray upside down and gently striking it on an absorbent paper. This also applies to all other wash steps.
9. Wash each strip once with 1 ml of Rinse Solution (RIN) for 1 minute on shaking platform/TwinCubator® (pour out RIN after incubation).
10. Add 1 ml of diluted Conjugate (see above) to each strip and incubate for 30 min on shaking platform/TwinCubator®.
11. Remove solution and wash each strip twice for 1 minute with 1 ml of Rinse Solution (RIN) and once for 1 minute with approx. 1 ml of distilled water (e. g. use wash bottle) on shaking platform/TwinCubator® (pour out solution each time). Make sure to remove any trace of water after the last wash.
12. Add 1 ml of diluted substrate (see above) to each strip and incubate protected from light without shaking. Depending on the test conditions (e. g. room temperature), the substrate incubation time can vary between 3 and 20 minutes. Extended substrate incubation times can lead to increased background staining and might impair interpretation of the results.
13. Stop reaction by briefly rinsing twice with distilled water.
14. Using tweezers, remove strips from the tray and dry them between two layers of absorbent paper.

Evaluation Examples

Examples for banding patterns and their evaluation with respect to fluoroquinolones (FQ), amikacin-kanamycin and capreomycin (AG/CP) and/or ethambutol (EMB) resistance

If all wild type bands display a signal, this is classified as positive and marked in the WT column of the respective gene as “+”. If at least one of the wild type bands is absent, this is classified as negative and marked in the WT column of the same gene as “–”. Negative entries are only made to the mutation columns when none of the mutation bands display a coloration. If at least one of the mutation bands display coloration, this is classified as mutation-positive.

Example 1 shows the wild type banding pattern. All wild type probes display a signal, but none of the mutations probes; hence, the evaluation chart has a “+” in the three wild type columns and a “–” in the three mutation columns. Accordingly, the boxes for fluoroquinolones (FQ), amikacin-kanamycin and capreomycin (AG/CP) and ethambutol (EMB) are marked as sensitive.

Example 2 shows the resistance pattern. One of the gyrA, rrs and embB wild type probes is missing. Hence, the boxes for gyrA WT, rrs WT and embB WT are marked with a “–”. As a mutation probe is developed, these boxes are marked with a “+”. The strain is evaluated as FQ, AG/CP and EMB resistant.

Example 3 and 5 show two FQ and EMB resistance patterns. One of the gyrA and embB wild type probes are missing. Hence, the boxes for gyrA WT and embB WT are marked with a “–”. In Example 3, as a mutation probe is developed, these boxes are marked with a “+”. The rrs profile does not deviate from the wild type pattern. The strains are evaluated as FQ and EMB resistant and AG/CP sensitive.

Example 4 shows a FQ resistance pattern. One of the gyrA wild type probe is missing. Hence, the box for gyrA WT is marked with a “–”. As a mutation probe is developed, this box is marked with a “+”. The rrs and embB and profile do not deviate from the wild type patterns. The strain is evaluated as FQ resistant and AG/CP and EMB sensitive.