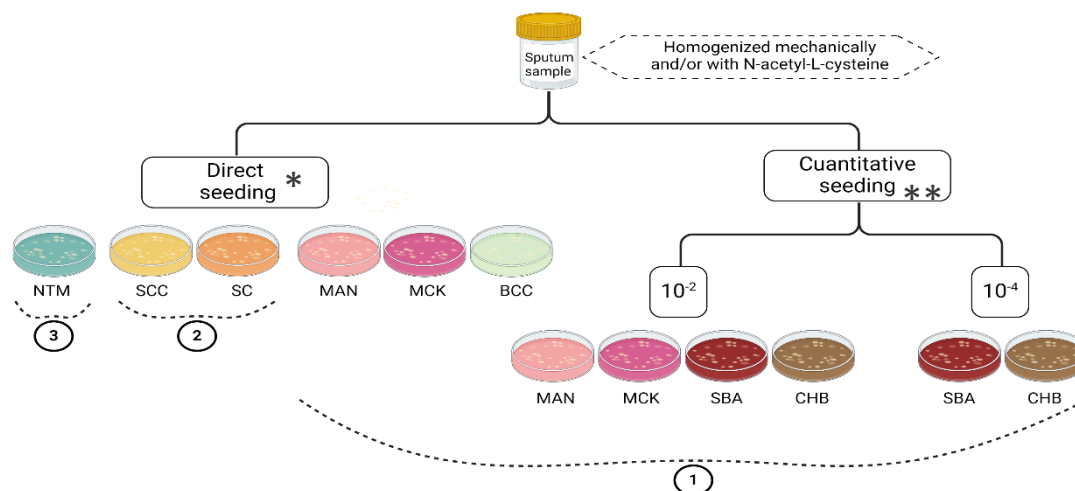


S1. Sputum seeding procedure used in our center.



- 1- At 37°C for 48h plus 5 days at room temperature; CHB at 37°C for 48h, in a CO₂ atmosphere.
- 2- At 30°C for 48h plus 10 days at room temperature.
- 3- At 37°C for 28 days.

* 50 µL of direct sample in each culture medium.

** Serial dilutions are performed with 50 µL of the sample in 4.95 mL of sterile saline solution (10⁻²). The 10⁻⁴ dilution is prepared with 50 µL of the 10⁻² dilution in 4.95 mL of sterile saline solution. Finally, 50 µL of each dilution were used to inoculate each corresponding plate.

NTM: Non-tuberculous mycobacteria agar; SC: Sabouraud dextrose agar with chloramphenicol; SCC: Sabouraud dextrose agar with chloramphenicol and cycloheximide; Man: Mannitol agar; MCK: MacConkey agar; BCC: *Burkholderia cepacia* Selective agar; SBA: Columbia-agar with sheep-blood; CHB: Columbia-agar with chocolate horse-blood plus bacitracin. Created with BioRender.com.

S2. Calculation of the number of colonies per milliliter

To calculate the CFU/mL in the original sample, the following formula was used:

$$N = n * \frac{V_t}{V_s} * 20 * D$$

N: total number of CFU/mL.

n: number of colonies counted on a specific culture medium.

V_t: total volume, corresponding to the initial volume of the sample (V_s) + volume of N-acetylcysteine required for homogenization.

20: Correction factor associated with seeding of 50 µL per plate (the count is reported per mL of sample).

D: Dilution factor. Correction factor associated with dilutions 1/1; 1/100 and 1/10,000 for quantitative culture.