Culture and Detection of *Mycobacterium tuberculosis* (MTB) and *Mycobacterium bovis* (BCG)

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[Abstract] *Mycobacterium tuberculosis* (MTB) is the bacterial pathogen responsible for tuberculosis, a human pulmonary infectious disease. *Mycobacterium bovis* (BCG) is the causative agent of tuberculosis in cattle, and is often used as the vaccine stain in humans. Specific recipes and methods for culture of MTB and BCG are described in this protocol.

Materials and Reagents

1. Middlebrook 7H9 broth base powder (Sigma-Aldrich, catalog number: M0178)
2. Middlebrook 7H11 agar base powder (Sigma-Aldrich, catalog number: M0428)
3. Middlebrook OADC growth supplement (Sigma-Aldrich, catalog number: M0678)
4. BD TB quick stain kit (BD Biosciences, catalog number: 212522)
5. General chemicals (Sigma-Aldrich)
6. ADNaCl (see Recipes)
7. 7H9 liquid medium (see Recipes)
8. 7H11 agar plates (see Recipes)

Equipment

1. T75 tissue culture flasks (Sigma-Aldrich, catalog number: Z707546)
2. Standard glass microscope slides
3. Light microscope
4. Bunsen burner or fixed flame source
5. Standard sterile petri dishes

Procedure

A. Culture
   1. Preparing 7H9 liquid medium

2. Day 1: Resuspend 1 frozen vial of 1 ml (450 million) MTB or BCG (stored in 15% glycerol and 85% 7H9 liquid medium) in 20 ml 7H9 liquid medium in a T75 flask.
3. Culture horizontally in an incubator humidified at 37 °C without CO_2 for 5 - 14 days.
4. Measure the OD_{600} every 5 days, till OD_{600} reaches 2.0.
5. Viable MTB/BCG colonies can be counted by plating bacterial suspensions at different dilutions on Middlebrook 7H11 agar plates supplemented with OADC, and then counting colonies after two weeks.
6. Harvest the bacteria through spin-down at room temperature, 3,000 *x g* for 7 min.

B. Detection: Ziehl-Neelsen acid-fast staining detection procedure
1. Pipet 10 µl MTB/BCG culture on a glass microscope slide and heat on top of a Bunsen flame until it is completely dry to fix the bacteria.
2. Flood the slide with carbol fuchsin stain (BD kit reagent A).
3. Heat the slide gently until it steams (5 min).
4. Pour off the carbol fuchsin.
5. Wash slide thoroughly with tap water (5 min).
6. Decolorize with acid-alcohol (5 min).
7. Wash slide thoroughly with tap water (5 min).
8. Flood slide with methylene blue (BD kit reagent B) counterstain (1 min).
9. Wash with tap water.
10. Blot excess water and dry in hand over Bunsen flame.
11. The slide is now ready to observe under a standard light microscope.

Recipes

1. ADNaCl
   15 g BSA
   6 g Dextrose
   2.55 g NaCl
   Dissolve in 300 ml ddH_2O
   Note: BSA will take some time to go into solution. Filter to sterilize the supplement and store covered in foil at 4 °C. Do not store for more than one month.
2. 7H9 liquid medium
   Dissolve 4.7 g of 7H9 powder in 900 ml dH_2O
   Add 2 ml glycerol and mix well
   Autoclave and let cool completely
   Store medium at room temperature in the dark

Note: Do not use medium that has been stored for longer than a month.

Right before use, add 2.5 ml 20% Tween 80 (filter sterilized) and 10% ADNaCl supplement. At this point the media can be filter sterilized or used directly. Media to which the supplement and Tween have been added should be stored at 4 °C and used within a few weeks.

3. 7H11 agar plates

   Dissolve 21 g of 7H11 powder in 900 ml dH_2O

   Add 5 ml glycerol and swirl to obtain a smooth suspension

   Note: Boil if necessary to completely dissolve the powder.

   Autoclave at 121 °C for 15 min

   Add 100 ml Middlebrook OADC Enrichment and 2.5 ml 20% Tween 80 (filter sterilized) to the medium when cooled to 50-55 °C

   Mix well and pour to make agar plates

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