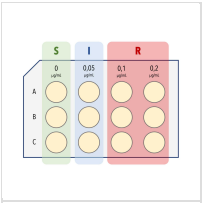

May 20, 2022

Preparation and use of 12-well plates for the rapid detection of terbinafine-resistant dermatophytes

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This protocol describes the steps necessary for the preparation and use of screening plates for the rapid detection of terbinafine resistant dermatophytes.

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1) When you take the medium out of the autoclave, be as quick as possible to add the terbinafine and transfer the medium to the plate wells. Since the medium contains agar, it will solidify fairly quickly.

2) Work in sterile conditions as much as possible. The medium is very easy to contaminate and can, if so, falsify the results.

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Terbinafine preparation 13m

13m

1 Weigh **16.33 mg** of Terbinafine hydrochloride **Merck Millipore Sigma Catalog #T8826** and add to an erlenmeyer flask containing **5 mL** of Dimethyl sulfoxide **Merck Millipore Sigma Catalog #D4540** . Mix without heating for **00:10:00** to dissolve. The final concentration of this terbinafine preparation is **3200 µg/mL** . Do not forget to add a magnetic bar. Cover each flask with glass wool and aluminium foil.

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Heated magnetic stirrer
AG602
LMR **AG602**

2 Autoclave this flask at **121 °C** for **00:30:00** 30m

Autoclave
Tuttnauer **3850EL**

3 Under hood, transfer **1 mL** of this preparation into 2 mL eppendorf tubes and store at **-80 °C** for later use. 5m

Biological safety cabinet
AC2-4S8-TU
Esco **303110**

Medium preparation 4h 25m

4 Dissolve **30 g** of 8m

Sabouraud dextrose broth **Merck Millipore**
Sigma Catalog #S3306

in **1 L** of

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MilliQ water **Contributed by users** and let mix on the heated magnetic stirrer for **00:05:00** (temperature and mixing speed knob at mid-step)

Heated magnetic stirrer
AG602
LMR **AG602**

5 12m

Difco granulated agar **Fisher**

Add **15 g** of **Scientific Catalog #BD 214530** and let dissolve for **00:10:00** while mixing and heating

6 5m

Pour **250 mL** of this medium into 4 erlenmeyer flasks and label each with final terbinafine concentrations (0.2 µg/mL for the first flask, 0.1 µg/mL for the second, 0.05 µg/mL for the third and 0 µg/mL for the last flask). Given the uncertainty of measurement, it is advisable to work from the highest concentration to the lowest. Do not forget to add a magnetic bar. Cover each flask with glass wool and aluminium foil.

7 4h

Autoclave these 4 flasks at **121 °C** for **00:30:00**

Autoclave
Tuttnauer **3850EL**

Plates preparation 1h 11m

8 2m

For the first flask (0.2 µg/mL) : add **15.6 µL** of a 3200 µg/mL terbinafine preparation (see

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section number 1). Let mix while heating (temperature and mixing speed knob at mid-step) for **00:02:00** . Caution : do not leave for more than 2 minutes otherwise the medium will solidify.

9 For the second flask (0.1 µg/mL) : add **7.8 µL** of a 3200 µg/mL terbinafine preparation (see section number 1). Let mix while heating (temperature and mixing speed knob at mid-step) for **00:02:00** . Caution : do not leave for more than 2 minutes otherwise the medium will solidify. 2m

10 For the third flask (0.05 µg/mL) : add **3.9 µL** of a 3200 µg/mL terbinafine preparation (see section number 1). Let mix while heating (temperature and mixing speed knob at mid-step) for **00:02:00** . Caution : do not leave for more than 2 minutes otherwise the medium will solidify. 2m

11 For the last flask (0 µg/mL) : does not contain terbinafine.

12 In a 12-well plate (3 rows and 4 columns), the first well of each row does not contain terbinafine. It serves as a growth control. Using an automated pipettor, add **3 mL** of the medium that does not contains terbinafine (flask number 4) to all the first wells of the plate. 15m

Motorized Pipette Filler
Levo Plus
DR. LAB 7033100100

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Microwell plate
Thermo Scientific 130185

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13 The second wells in each row contain a concentration of 0.05 µg/mL of terbinafine. Using an automated pipettor, add **3 mL** of the medium that contains **0.05 µg/mL** of terbinafine (flask number 3) to all the second wells of the plate. 15m

Motorized Pipette Filler
Levo Plus
DR. LAB 7033100100

Microwell plate
Thermo Scientific 130185

14 The third wells in each row contain a concentration of 0.1 µg/mL of terbinafine. Using an automated pipettor, add **3 mL** of the medium that contains **0.1 µg/mL** of terbinafine (flask number 2) to all the third wells of the plate. 15m

Microwell plate
Thermo Scientific 130185

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Motorized Pipette Filler
Levo Plus
DR. LAB 7033100100

15 The last wells in each row contain a concentration of 0.2 µg/mL of terbinafine. Using an automated pipettor, add **3 mL** of the medium that contains **0.2 µg/mL** of terbinafine (flask number 1) to all the last wells of the plate. 15m

Motorized Pipette Filler
Levo Plus
DR. LAB 7033100100

Microwell plate
Thermo Scientific 130185

16 Cover the plates with their lids and let the medium solidify at room temperature. Label the plates with the corresponding terbinafine concentrations for each well and store at **4 °C** for future use. 5m

Use of the plates

17 Using a sterile swab, prepare a suspension (sterile water) 0.5 McF of the dermatophyte to be

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studied.

18 Transfer **25 µL** of this suspension to each of the 4 wells of the plate.

19 Annotate the plate with the number of the studied strains and incubate at 30°C for 96h before reading the result.

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