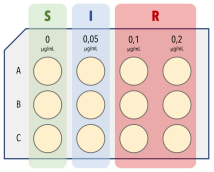
May 20, 2022

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

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**Protocol status:** In development
**We are still developing and optimizing this protocol**

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**Keywords:** terbinafine resistant dermatophyte, resistant dermatophyte, well plates for the rapid detection, screening plate, plates for the rapid detection, terbinafine, rapid detection, well plate

## Disclaimer



## Abstract

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

## Guidelines

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.

## Protocol materials

- Sabouraud dextrose broth **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S3306
- MilliQ water
- Difco granulated agar **Fisher Scientific** **Catalog #**BD 214530
- Terbinafine hydrochloride **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**T8826
- Dimethyl sulfoxide **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**D4540

## Terbinafine preparation

13m

1 13m

Weigh 16.33 mg of Terbinafine hydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T8826 and add to an erlenmeyer flask containing 5 mL of Dimethyl sulfoxide **Merck MilliporeSigma (Sigma-Aldrich) 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.

Equipment

Heated magnetic stirrer

| | |
|---|---|
| NAME | Heated magnetic stirrer |
| TYPE | AG602 |
| BRAND | LMR |
| SKU | AG602 |

2 30m

Autoclave this flask at 121 °C for 00:30:00

Equipment

| | |
|---|---|
| Autoclave | NAME |
| Tuttnauer | BRAND |
| 3850EL | SKU |
| https://tuttnauer.com/laboratory-autoclaves/laboratory-research/benchtop-autoclaves/el-d-line | LINK |

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

Equipment

| | |
|---|---|
| Biological safety cabinet | NAME |
| AC2-4S8-TU | TYPE |
| Esco | BRAND |
| 303110 | SKU |
| https://analis.be/fr-be/sample-handling-in-ivf-labs/ac2-4s8-tu-airstream-plus-class-ii-biological-303110 | LINK |

## Medium preparation 4h 25m

4 Dissolve 30 g of 8m

Sabouraud dextrose broth **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S3306

in 1 L of MilliQ water and let mix on the heated magnetic stirrer for 00:05:00 (temperature and mixing speed knob at mid-step)

Equipment

| | |
|---|---|
| Heated magnetic stirrer | NAME |
| AG602 | TYPE |
| LMR | BRAND |
| AG602 | SKU |

5 Add 15 g of Difco granulated agar **Fisher Scientific Catalog #**BD 214530 and let dissolve for 00:10:00 while mixing and heating 12m

6 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. 5m

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

Equipment

| NAME | BRAND | SKU | LINK |
|---|---|---|---|
| Autoclave | Tuttnauer | 3850EL | https://tuttnauer.com/laboratory-autoclaves/laboratory-research/benchtop-autoclaves/el-d-line |

## Plates preparation

1h 11m

8 For the first flask (0.2 µg/mL) : add 15.6 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

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 15m

the medium that does not contains terbinafine (flask number 4) to all the first wells of the plate.

Equipment

| | |
|---|---|
| Motorized Pipette Filler | NAME |
| Levo Plus | TYPE |
| DR. LAB | BRAND |
| 7033100100 | SKU |
| http://www.drlab.co/index.php?route=product/product&product_id=177 | LINK |

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Equipment

| | |
|---|---|
| Microwell plate | NAME |
| Thermo Scientific | BRAND |
| 130185 | SKU |
| https://www.fishersci.com/shop/products/biolite-multidishes-microwell-plates/12556005 | LINK |



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

Equipment

| | |
|---|---|
| Motorized Pipette Filler | NAME |
| Levo Plus | TYPE |
| DR. LAB | BRAND |
| 7033100100 | SKU |
| http://www.drlab.co/index.php?route=product/product&product_id=177 | LINK |

Equipment

| | |
|---|---|
| Microwell plate | NAME |
| Thermo Scientific | BRAND |
| 130185 | SKU |
| https://www.fishersci.com/shop/products/biolite-multidishes-microwell-plates/12556005 | LINK |



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

Equipment

| | |
|---|---|
| Microwell plate | NAME |
| Thermo Scientific | BRAND |
| 130185 | SKU |
| https://www.fishersci.com/shop/products/biolite-multidishes-microwell-plates/12556005 | LINK |



Equipment

| | |
|---|---|
| Motorized Pipette Filler | NAME |
| Levo Plus | TYPE |
| DR. LAB | BRAND |
| 7033100100 | SKU |
| http://www.drlab.co/index.php?route=product/product&product_id=177 | LINK |

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

Equipment

| | |
|---|---|
| Motorized Pipette Filler | NAME |
| Levo Plus | TYPE |
| DR. LAB | BRAND |
| 7033100100 | SKU |
| http://www.drlab.co/index.php?route=product/product&product_id=177 | LINK |

Equipment

| | |
|---|---|
| Microwell plate | NAME |
| Thermo Scientific | BRAND |
| 130185 | SKU |
| https://www.fishersci.com/shop/products/biolite-multidishes-microwell-plates/12556005 | LINK |



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 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.