TD test protocol 9/26/19

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[1]In-house protocol

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

Tolerance detection test
Bikash(Adapted from(Gefen et al. 2017), Scientific reports)

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Gefen, O., Chekol, B., Strahilevitz, J., and Balaban, N.Q. 2017. TDtest: easy detection of bacterial tolerance and persistence in clinical isolates by a modified disk-diffusion assay. Scientific reports 7, p. 41284.
Hudzicki, J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol.

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GUIDELINES

- Bacterial Strains
- Media and Reagents
- Preparation of Disks
- TD test: Day 0 - Day 3

MATERIALS TEXT

Media and Reagents:

1. Muller Hinton agar
2. Antibiotics
3. 6 mm filter paper disc (BBL^{TM} 231039 from BD$^{\#}$)
4. Determine antibiotic concentration to be tested (can screen with MIC or lower concentration of antibiotics against the organism you are testing to yield a large zone of inhibition (at least >= 15 mm)
5. Glucose (20% stock)

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BEFORE STARTING

Bacterial strains:- Determine your replicates and controls necessary for the test and either a) overnight culture plates OR b) stationary phase liquid culture (start this on Day 0)

Preparation of disks:

- Use the BBL 231039 empty discs(OR cut the Filter paper (Whatman, #1) in circles of 6 mm diameter, sterilize by autoclave)and impregnate witheither4–10 µlof antibiotic solution to achieve the final required antibiotic concentration (see example below, prepare or dilute your stock solution as required)and leave to dry at room temperature.

Antibiotic	Stock	Requires	Add to disc	Remarks
Ampicillin	25 mg/mL	250 ug	10 ul	
Ciprofloxacin	0.01 mg/mL	0.1 ug	10 ul	
Kanamycin	1 mg/mL	10 ug	10 ul	
Chloramphenicol	1 mg/mL	10 ug	10 ul	
Gentamicin	1 mg/mL	10 ug	10 ul	

- To prepare glucose disc, add 2mg (10 ul of 20% Glucose) onto the filter paper disc and leave to dry at room temperature. Alternatively, you can add glucose to the antibiotic disc on Day 2.

TD test: Step 1 - Day 1

1 Preparation of Mueller-Hinton plate (Hudzicki n.d.)

1.1 Allow an MH agar plate (one for each organism to be tested) to come to room temperature. It is preferable to allow the plates to remain in the plastic sleeve while they warm to minimize condensation.

1.2 If the surface of the agar has visible liquid present, set the plate inverted, ajar on its lid to allow the excess liquid to drain from the agar surface and evaporate. Plates may be placed in a 37°C incubator or

in a laminar flow hood at room temperature until dry (usually 10 to 30 minutes).

1.3 Appropriately label each MH agar plate for each organism to be tested

2 Inoculation of the MH plate

2.1 Transfer 2 ml of stationary phase culture into the inoculum tube. Do not transfer any cell debris. Dip a sterile swab into the inoculum tube.
- Alternatively, take a colony grown overnight on appropriate plates, mix with 0.5 mL saline, and dip a sterile swab into this inoculum tube.

2.2 Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. The swab should not be dripping wet.

2.3 Inoculate the dried surface of an MH agar plate by streaking the swab three times over the entire agar surface; rotate the plate approximately 60 degrees each time to ensure an even distribution of the inoculum.

2.4 Rim the plate with the swab to pick up any excess liquid.

2.5 Discard the swab into an appropriate container

2.6 Leaving the lid slightly ajar, allow the plate to sit at room temperature at least 3 to 5 minutes, but no more than 15 minutes, for the surface of the agar plate to dry before proceeding to the next step.

3 Placement of antibiotic discs

3.1 Place the appropriate antimicrobial-impregnated disks on the surface of the agar, using either forceps to dispense each antimicrobial disk one at a time

3.2 Once all disks are in place, replace the lid, invert the plates, and place them in a 35°C air incubator for 16 to 18 hours. When testing Staphylococcus against oxacillin or vancomycin, or Enterococcus against vancomycin, incubate for a full 24 hours before reading.

3.3 You should avoid placing disks close to the edge of the plate as the zones will not be fully round and can be difficult to measure.

3.4 Each disk must be pressed down with forceps to ensure complete contact with the agar surface or irregular zone shapes may occur.

3.5 If the surface of the agar is disrupted in any way (a disk penetrating the surface, visible lines present due to excessive pressure of the swab against the plate during inoculation, etc.) the shape of the zone may be affected.

TD test: Step 2 - Day 2

4 Replace the antibiotic disk with a glucose (2 mg) disk after 18 hours and incubate the plate for an additional overnight.

Alternatives for this step:
- Add the glucose solution directly on the antibiotic disk, instead of replacing it.
- Use other nutrients instead of glucose.

Step 2 - Day 3

5 Result: Note the difference in the zone of inhibition and/or the number of tolerant colonies on the zone of inhibition. Use the imaging system in the photodoc room (if needed talk to HGB lab to operate the machine) to take images of the plates (export images as tif or png file).