iGEM2016 – Microbiology – BMB – SDU	
Title: DISC diffusion assay **SOP number:** SOP0029_v01 **Version number:** 01	**Date issued:** 2016.09.27 **Review date:** 2016.09.27 **Written by:** Cathrine Høyer

1. Purpose

To assay the susceptibility of antimicrobial compounds against chosen bacterial strains

2. Area of application

This procedure is valid for *all bacterial strains*

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Refrigerator	Laboratory (class 1)	•	
Small autoclave	Laboratory (class 1) – V18-405-0	•	121°C
Pipette (p1000, 100)		•	
Measuring pitcher 1 L	Glass hallway	•	

4. Materials and reagents – their shelf life and risk labelling

Name	Components	Supplier / Cat. #	Room (hallway storage)	Safety considerations
Blue pipette tips		Contact lab-manager	Micro storage	
Green pipette tips		Contact lab-manager	Micro storage	
Blue pipette tips		Contact lab-manager	Micro storage	
Difco Agar			Autoclave room	
Mueller-Hinton			Autoclave room	
Appropriate antibiotic	Manufacture/cat#, stock solution conc.; Stock solution date of		V18-405-0	

	creation, dilution in medium.			
Plastic Petri dishes			Contact lab-manager (Sarstedt)	
Saline solution	Sodium chloride 8.5 g Water 1000 L		Autoclave room	
Filter paper disks for antibiotic assay (6 mm), sterile			Micro storage	

5. QC – Quality Control

Calculation of required volume of antibiotic by;

$$C_1 \cdot V_1 = C_2 \cdot 10\mu L$$

It is possible to leave the autoclaved MH media at 58°C up till 24 hours before pouring into plates.

6. List of other SOPs relevant to this SOP

JMJ_SOP00012_v01_TK_Small autoclave
SOP0025_v01_Impact_Purification

7. Environmental conditions required

8. Procedure

Preparation of Mueller-Hinton Agar plates:

8.1.Mix components of Mueller-Hinton and Agar in a glass bottle
- 8.1.1 add 21 g of Mueller-Hinton + 17 g of Agar to a 1 L glass bottle
- 8.1.2 add 1000 mL desterilized water
- 8.1.3 Mix with a magnetic stirrer for 2 minutes

8.2 Autoclave the Mueller Hinton-Agar
8.3 When the temperature is between 50-60 °C fill up the required amount of petri dishes
8.4 Dry on table for at least 20 minutes
8.5 Note type and concentration of antibiotic and the date at the bottom of the dishes
- 8.5.1 If stored, place in bag in the refrigerator until use

Inoculation of Mueller-Hinton plate:

8.6 Add 4 mL saline solution to a 15 mL falcon tube
8.7 Touch the top of a isolated colony of the appropriate bacterial strain, with a pipette tip and transfer the colony to the falcon tube with saline. Emulsify the inoculum on the inside of the tube to avoid clumping of the cells.
8.7.1 Adjust the inoculum turbidity to a standard of 0.5 McFarland. McFarland 0.5 equals approximately 10^8 CFU/mL
8.8 Within 15 minutes of preparing the adjusted inoculum, dip a sterile cotton swab into the inoculum. Streak the swab over the entire surface of the Mueller Hinton agar plate.

8.9 Allow any excess moisture on the agar surface to be absorbed prior to applying the antimicrobial disks. The lid of the plate may be left ajar for 3-5 minutes (no more than 15 minutes) to allow any excess moisture to be absorbed before applying disks.

8.9.1. Verifying Purity of Inoculum:
8.9.2. Plate 10 µL of the inoculum from point 8.7.1. to a plain Mueller Hinton Agar plate (or other non-selective media) as a control
8.9.2 Incubate plates inverted at 36±1 °C for 16 to 18 hours in ambient air.

Dispensing Antimicrobial Disks:

8.10 Dispense disks to the agar surface with a disk dispenser or sterile forceps (forceps can be sterilized by flaming with alcohol). Do not relocate any disks after contact with the agar.
8.10.1 Note that there is a mark on the edge of the bottom half of the plate to allow orientation.
8.12. Prepare standard solutions containing 0.5, 1, 2, 4, 10 and 20 µg/mL of antimicrobial compound.
8.13. Add 10 µL of the diluted antimicrobial solutions to the center of the disk (one disk per concentration) as stated in figure 1 (only for example).
8.14 Incubate overnight at 37 °C, with the agar-containing portion at the bottom.

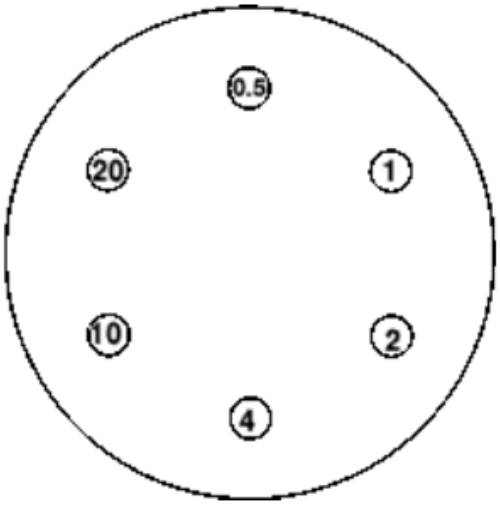

Figure 1

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z…)	Remarks
ON Culture		Liquid bacterial waste	
Mueller-Hinton agar rest		GMO yellow waste	

10.Time consumption

- Total-time 2 days.
- Hands-on-time 1-3 hours.

11. Scheme of development

Date / Initials	Version No.	Description of changes
16.09.27 / CHC	01	The SOP has been written
	01	The SOP has been approved

12.Appendices

Composition and preparation of culture media and reagents

Mueller Hinton Agar

- 21 g of Mueller-Hinton component
- 17 g of Agar
- 1000 mL of desterilized water

Saline solution

- Sodium chloride 8.5g
- Water 1000 mL

Preparation: Dissolve the sodium chloride in the water by heating if necessary. Adjust pH to 7.0. Dispense the solution into tubes so 4 mL is obtained after autoclaving at 121°C for 20 min

Dilution of antimicrobial compound

- X µL Antimicrobial compound + X µL desterilized water = total volume of 10 µL