

Evidence of the effect of antibiotics, chemotherapeutics, and disinfectants (Item No.: P4140500)

Curricular Relevance



Difficulty



Intermediate

Preparation Time



30 Minutes

Execution Time



2 Hours

Recommended Group Size



2 Students

Additional Requirements:

- Antibiotics
- Chemotherapeutics
- Disinfectants

Experiment Variations:

Keywords:

Disinfection, Sterility, Antibiotics, Chemotherapeutics, Disinfectants, Nutrient agar

Overview

Short description

Principle

Evidence concerning the effect of substances inhibiting the growth of microorganisms, e.g. antibiotics, chemotherapeutics, and disinfectants, can be provided by treating cultures with the substances to be studied and by observing the growth of the microorganisms. Evidence concerning the effect of disinfectants can be provided by letting sterile agar in Petri dishes become infected with airborne microorganisms, spraying the plates with the disinfectant to be studied, and by incubating the plates. This method can also be applied in order to demonstrate and compare the effect of different concentrations of the same disinfectant in a manner that is usually sufficient for school purposes. Evidence concerning the effect of antibiotics and chemotherapeutics can be provided by way of the so-called punched-hole test.



Fig. 1: Procedure.

Equipment

Position No.	Material	Order No.	Quantity
1	Tripod, ring d=140 mm, h=240 mm	33302-00	1
2	Glass beaker DURAN®, tall, 600 ml	36006-00	1
3	Graduated cylinder 100 ml	36629-00	1
4	Graduated pipette, 1 ml	36595-00	1
5	Graduated pipette 10 ml	36600-00	1
6	Water, distilled 5 l	31246-81	1
7	Wire gauze with ceramic, 160 x 160 mm	33287-01	1
8	Bottle, nar. mouth, 100ml, clear, p.st	41101-01	4
9	Safety gas tubing, DVGW, sold by metre	39281-10	1
10	Bunsen burner, natural gas, w. cock	32167-05	1
11	Petri dish, d 100 mm	64705-00	10
12	Autoclave with insert	04431-93	1
13	Formaldehyde sol. ca. 35% 500 ml	48146-50	1
14	Compact Balance, OHAUS TA 302, 300 g / 0.01 g	49241-93	1
15	Microscopic slides, 50 pcs	64691-00	1
16	Test tube rack for 12 tubes, holes d= 22 mm, wood	37686-10	1
17	Erlenmeyer flask, narrow neck, 500 ml	36121-00	2
18	Test tube, 160 x 16 mm, 100 pcs	37656-10	1
19	Peptone, dry, from meat 50 g	31708-05	1
20	Spatula, double blade, 150 mm	33460-00	1
21	Glass rod, boro 3.3, l=300mm, d=7mm	40485-05	1
22	pH test sticks 6.5-10, 100 sticks	30301-04	1
23	Sodium hydroxide, pellets, 500 g	30157-50	1
24	Agar-agar, powdered 100 g	31083-10	1
25	Sterile stoppers f. id 15mm, 250	39266-00	1
26	Sterile stoppers f. id 29mm, 100	39267-00	1
27	Pipettor	36592-00	1
28	Universal oven, 32 liters, 230 V	49559-93	1
29	Autoclavable disposable bag, PA	46428-04	1
30	Pressure aerosol can, 125 ml	35006-00	1

Tasks

1. Evidence of the effect of antibiotics and chemotherapeutics
2. Evidence of the effect of disinfectants

Set-up and procedure

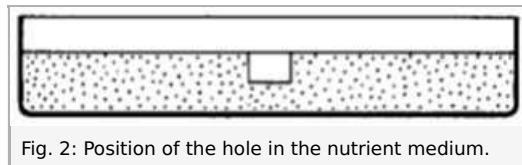
Procedure

Evidence of the effect of antibiotics and chemotherapeutics

Preparation of the nutrient media: see P4140100.

After the solidification of the nutrient medium in the dishes, use a cork borer with a diameter of 8 mm that has been sterilised in the flame of a gas burner beforehand and carefully bore a hole into the middle of the plate. The depth of this hole should be approximately half the thickness of the nutrient medium layer (Fig. 2). Ensure that no cracks or fissures form in the nutrient medium.

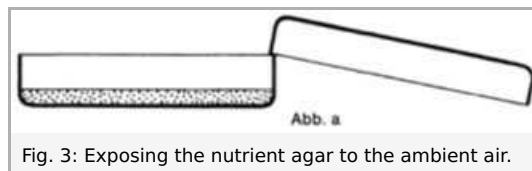
Inoculate the plate with several different types of bacteria by smearing them in streaks from the edge to the hole. The bacteria can be taken from a plate on which bacteria have grown. Pipette a solution of the antibiotic or chemotherapeutic that is to be tested into the hole. The solution will then diffuse into the nutrient medium from all sides. Incubate the plates at 30°C in the incubator.



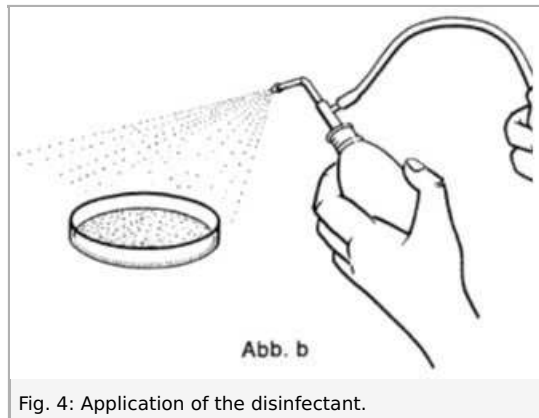
Evidence of the effect of disinfectants

Preparation of the nutrient media: see P4140100.

After the solidification of the nutrient medium in the dishes, expose four plates to an infection with microorganisms from the ambient air by leaving the dishes open. Place the covers in a tilted manner on the rim of the lower part (Fig. 3).



After 30 minutes, spray a thin layer of the disinfectant to be studied with the aid of an atomiser onto the surface of the nutrient medium in two plates (Fig. 4). Possible disinfectants are a 3% formaldehyde solution, Sagrotan, Lysoform, etc., for example.



If the disinfectant can be purchased only as a parent solution, it must be diluted with water. If this is the case, follow the instructions that are enclosed with the disinfectant precisely. Close all four plates with their respective covers and incubate them at room temperature. Position the sprayed and unsprayed plates at a certain distance from each other in order to absolutely avoid that traces of the disinfectant that evaporates from the sprayed nutrient medium penetrate the unsprayed plates. This is also the reason why the plates are not incubated in an incubator.

Theory and evaluation

Result and evaluation

Evidence of the effect of antibiotics and chemotherapeutics

Within two to three days, the bacteria develop on the inoculation streaks. Depending on their resistance with regard to the antibiotic or chemotherapeutic, they grow more or less closely towards the hole with the test solution (Fig. 5).

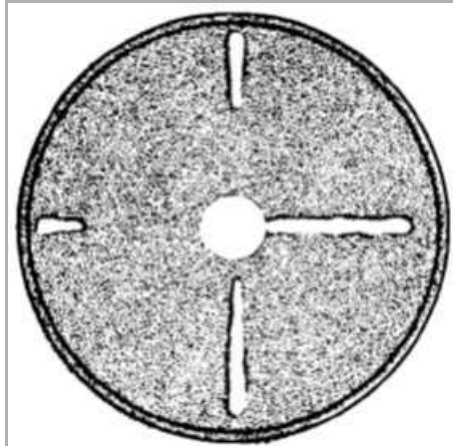


Fig. 5: Depending on their resistance with regard to the antibiotic or chemotherapeutic, the microorganisms grow more or less closely towards the hole with the test solution.

Evidence of the effect of disinfectants

Within one week, numerous colonies of microorganisms of various kinds develop on the nutrient medium that has not been treated with any disinfectant. On the disinfected nutrient medium, the development is significantly smaller or even completely suppressed depending on the effectiveness or concentration of the disinfectant.