# Antibiotic Agar Experiment (50 mins)

This activity is covered in the [KS4 resources](http://www.e-bug.eu/en-gb/ks4-antimicrobial-resistance) and involves an experiment using agar culture plates with indicators to grow microbial cultures and to test whether the microbes are killed by a range of antibiotics, and to decide which microbe is causing the illness and whether antibiotics are needed.

## Before you begin you will need:

* Gloves
* Petri dishes
* Base Agar
* Hot plate
* Phenol Red\*
* Wax Crayon/marker
* Disposable droppers
* Hydrochloric acid
* Cork borer
* Test tubes
* Test tube rack

## Use the introduction in the lesson plan to discuss:

* What participants think medicine is. Display the range of food and medicine on the counter. Explain that the term medicine has been defined as a substance or preparation affecting wellbeing, used in maintenance of health and prevention, alleviation or cure of disease.
* How the different items can be divided into two groups, one which they think is medicines and one which isn’t. The group will probably divide the items into commercial medication and food stuffs. Explain that many food stuffs can also have medicinal properties (honey can be used as an antibacterial agent – many people believe that honey helps cure a sore throat. Peppermint tea aids in digestion, ginger and garlic also have antibacterial properties, orange juice contains high quantities of vitamin C) and many commercial medicines are based on these food sources.
* Diet, and how a healthy diet, can help prevent us being ill and avoid having to visit the doctor, e.g., it is thought that regular intake of fruit and vegetables containing vitamin C can help reduce the chances of being ill with the common cold.
* How medicines should only be used for the illness for which they were intended. Ask the group what they think antibiotics should be used for. Highlight that antibiotics are ONLY used for bacterial infections and that they do not work on viral or fungal infections.
* A presentationis available on the discovery and resistance of antibiotics.

## Advance preparation:

The following preparation is for 1 group of 5 students

### Materials Required

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Base Agar |  | Petri dishes |  | Hydrochloric acid |
|  | Hot plate |  | 20 Test tubes |  | Disposable droppers |
|  | Phenol Red |  | 5 Test tube racks |  | Cork borer |
|  | Wax Crayon/marker |  |  |  |  |

### Agar Plate Preparation

1. Make up 100ml of base agar following the manufacturer’s instructions.
2. When cooled slightly, but not solid, pour 1 agar plate (to demonstrate no growth). When complete add enough (~10 drops) 2 – 4% Phenol Red to turn the agar a deep red/dark orange and mix well.
3. Pour approx. 20ml into each petri dish and leave to cool.
4. When solidified, make 5 evenly spaced bore holes in each agar plate.
5. Label each petri dish with A, B, C, D

### Antibiotic (test-tube) Preparation

1. Set up a test tube rack of 5 test tubes for each patient. Label each test tube with one of the following labels

a. Penicillin b. Methicillin c. Erythromycin d. Vancomycin e. Amoxicillin

1. Transfer 5ml of the following solutions into the appropriately labelled test tube

a. Penicillin b. Methicillin c. Erythromycin d. Vancomycin e. Amoxicillin

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| A | Water | Water | Water | Water | Water |
| B | 10% HCl | 5% HCl | 1% HCl | 0.05% HCl | 5% HCl |
| C | Water | Water | 1% HCl | 0.05% HCl | Water |
| D | Water | 0.05% HCl | 0.05% HCl | 0.05% HCl | Water |

**NB: It is extremely important to have the correct concentrations of HCl (antibiotics) for each patient.**

### Set up a work bench for the group as follows:

a. Place the appropriate patient’s agar plate next to each corresponding rack of test tubes at 4 stations across the bench

1. A dropper for each test tube
2. A ruler with mm markings
3. It may be easier for students if they place each patient’s agar plate on a piece of white paper and label the paper next to each bore hole with the antibiotic name.

### Use the following steps as a guide to implement this activity:

1. This activity should be carried out in small groups of 3 - 5 participants.
2. A workbench should be set up for each group containing:
3. 4 agar culture plates with indicator, each labelled with a patient’s name.
4. 4 test tube racks, each containing 5 antibiotic solutions, one beside each agar plate.
5. Provide participants with a copy of SW 1 and SW 2.
6. Explain that Eva is working in a hospital lab and it is her job to grow microbial cultures from swabs taken from patients at a doctor’s surgery. Eva then tests whether the microbes are killed by a range of antibiotics. The results help the doctor decide what microbe is causing the illness and which antibiotics, if any, to prescribe.
7. Highlight that the red colour represents the microbes growing in the agar; it may help here to show them an agar plate with no indicator (yellow), i.e. no growth.
8. Place plates on a sheet of white paper. Participants should label each bore hole and drop antibiotics, one drop at a time, into the appropriately labelled hole until the hole is filled with the antibiotic.
9. Replace the lid of the petri dish and leave for 5 minutes.
10. After 5 minutes, participants should measure the size of the decolourised zone (inhibition) if present.
11. Participants should complete their worksheets in groups and discuss with the teacher.

**Use the plenary questions to check participant’s understanding after the activity is complete.**

worksheet to depict predicted results from the experiment. 
Patient A - change to any bore holes;
Patient B - zone of inhibition around all except methicillin
Patient C - zone of inhibition present only around Vancomycin
Patient D - zone of inhibition present around methicillin, erythromycin, and vancomycin 