MICROBIOLOGY:
Antibiotics and Bacteria

The Southern Biological Antibiotic Set consists of a petri dish containing either 10, 50, or 100 Mastrings. Each lobe of the Mastring is impregnated with a different antibiotic. The symbols indicate antibiotics as follows:

Symbol	AP	c	PG	S	ST	T
Name	Ampicillin	Chloramphenicol	Penicillin G	Streptomycin	Sulphatriad	Tetracycline
Colour	Grey	Green	Pink	White	Mauve	Brown

In order to test the sensitivity of a specific bacterial species to the antibiotics concerned, use a sterile swab to spread a broth culture of the test organism over the entire surface of a nutrient agar plate. For best results we recommend:

1. Use a separate nutrient agar plate for each strain of bacteria to be tested
2. Avoid using nutrient agar plates that contain excessive condensed water droplets shake the broth culture before applying it to the surface of the nutrient agar plate
3. Allow the coated nutrient agar plate to stand for 30 minutes after swabbing

Please note that results can be affected by variables such as temperature, moisture levels and concentration of bacteria. Run a control to validate bacterial growth in the absence of antibiotics.

Sterilize the tips of a pair of forceps by flaming in a Bunsen flame. Allow to cool. Transfer one Mastring to each of the coated nutrient agar plates, gently pressing the lobes of the disc onto the surface of the plates. Resterilize forceps in between testing different bacterial cultures. Incubate each plate at 37°C for 24 hours, or at room temperature for 3 days.

Zones of growth inhibition around any lobe of the Mastring denote the sensitivity of the organism to the antibiotic concerned. Organisms that show high sensitivity to most of the antibiotics may have their growth restricted to the very edge of the petri dish.

There are numerous strains (serotypes) of E. coli, the majority of which, including that from Southern Biological, are non pathogenic. All Risk Group 1 bacterial cultures sold by Southern Biological are generally considered to be non-pathogenic, although it is essential that students and teachers use and regard ALL bacteria as potentially dangerous. For this reason, all plates, swabs, cultures, etc., should be autoclaved or incinerated after use. If heat sterilization is not an option, use undiluted bleach on cultures prior to disposal. 70% alcohol should be made available for skin disinfection in the case of accidental contact with the cultures.

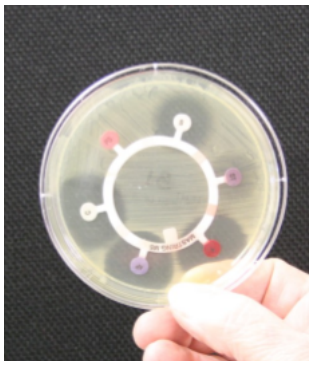
Q.A.C. (Quaternary Ammonium Compound)- disinfectant concentrate (industrial grade), similar to Zephiran, is available from Southern Biological in 100mL or 500mL volumes, and should be diluted 1:100 in water for use. This disinfectant is suitable for surfaces and discarded jars. Fresh solutions should be prepared each day. Minimum contact time for effective bactericidal activity is three minutes.

Results from our own laboratory testing:

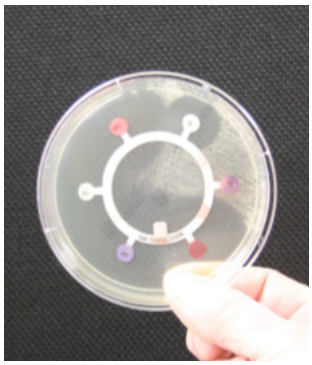
Organism	Code	Ampicillin	Chloramphenicol	Penicillin G	Streptomycin	Sulphatriad	Tetracycline
Escherichia coli	B1	++	+++	--	++	+	++
Staphylococcus epidermidis	B2	+++	+++	+++	++	--	+++
Micrococcus lutea	B3B	+++	+++	+++	+++	++	+++
Bacillus subtilis	B5B	+++	++	++	++	++	++

Symbol Key:

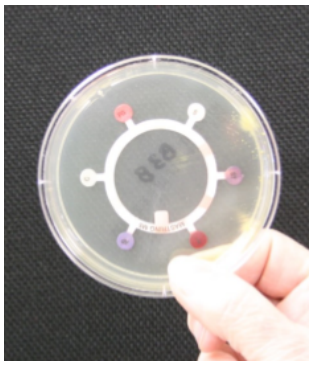
--	Bacteria is resistant to the antibiotic (no zone of resistance)
+	Bacteria is marginally susceptible to the antibiotic (small
++	Bacteria is susceptible to the antibiotic (medium zone)
+++	Bacteria is highly susceptible to the antibiotic (large zone)



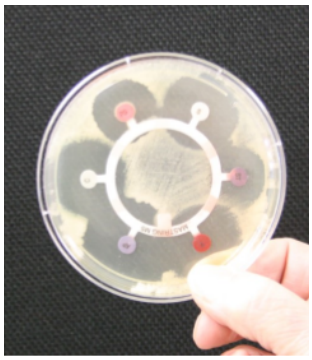
Escherichia coli



Staphylococcus epidermidis

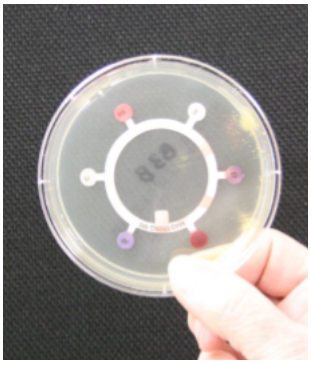


Micrococcus lutea



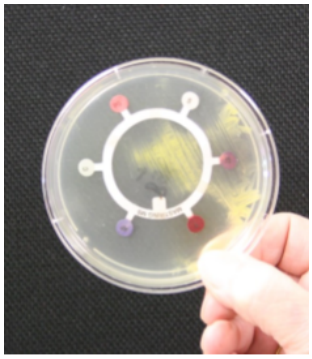
Bacillus subtilis

Example plates from our laboratory. Each plate was swabbed with the nominated bacteria and allowed to sit for 30 mins prior to the placement of the Mastring onto the surface. Incubation was at 37°C for 24 hours.



Micrococcus lutea

Mastring placement after 30 mins then 24 hr incubation.



Micrococcus lutea

Mastring placement after 6 hr incubation then followed with another 16 hr incubation.

Micrococcus lutea is highly suspectible to all the antibiotics on the Mastring. An experimental variation where the swabbed plate is incubated for 6 hours prior to placement of the Mastring and then incubated for a further 16 hours provided the following comparison.