Bacteria are found just about everywhere, and most of them are nonpathogenic. Others are just plain harmful, pathogenic forms.

Still others are harmless as long as they maintain their personal space, but become a threat when they get into areas other than their natural habitat.

E. coli, for example, are natural residents of large intestines. There they cause no harm and actually help by assisting with waste processing, vitamin K production, and food absorption.

When E. coli or some of the other types of microorganisms leave their normal habitats and enter areas where they are not normally found, they can cause disease.

Contamination of foods by E. coli or other microorganisms is a serious threat to health. How can we test for organisms such as E. coli that might cause microbial contamination?

What if we find that the organisms are present in some substances - how can we determine the degree of contamination of the material?

The rate of microbial spoilage depends upon the chemical composition of the affected substance(s) and the types of microorganisms causing the infection. Freezing, boiling and secure packaging help prevent contamination.

Improper handling, such as employees returning to processing areas from the bathroom without washing their hands, can cause serious contamination. Improper slaughter and packaging procedures can also cause contamination. Careless beef processing has apparently caused recent outbreaks of a lethal form of E. coli. Animal feces containing E. coli were included in beef processing along with the beef body tissues.

**EXERCISE #1**

Each member of a two-person team needs to obtain a clean, closed Petri dish that contains nutrient agar.

Each team needs to select one culture solution of an unknown organism. Make a note of the identification code on the unknown container. Keep the solution closed until it is time to use it.

Working with microbial cultures requires the use of aseptic technique to prevent the contamination of both the laboratory as well as its personnel.

All materials and media used for the growth of microbes must be sterilized prior to use. While working with the cultures, the spreaders, inoculation loops and other materials must be kept sterile by flaming them both before and after their use.

Culture tubes must be flamed when opened and also prior to closing.

Observe the location of the Bunsen burner on your lab table. You will use the burner flame to sterilize the opening of your unknown culture tube, the glass spreader and the wire inoculation loop.
Mark the outer bottom cover of the Petri dish (use tape or grease pencil) with your name.

Petri dishes must be stored upside down (agar hanging from the small lid) in the incubator, to prevent moisture from washing away the organisms growing on the surface of the nutrient agar.

Use this procedure for preparing a growth plate of the unknown solution by means of the spreader method:

- Turn the Petri dish right side up,
- Open the unknown culture tube, and flame its opening,
- Open the lid of the Petri dish only part way: just enough so that you can pour the unknown on the agar surface; make a puddle a little smaller than the size of a dime,
- Close the Petri dish,
- Flame the opening of the unknown culture tube and close it, then
- Take the glass elbow (called a spreader) from its container of alcohol, tapping as much alcohol as possible off its surface against the inside wall of its container,
- Carefully flame the elbow and hold it until it cools slightly,
- Open the Petri dish just enough to admit the glass elbow,
- Use the sterile spreader to spread the food solution evenly over the surface of the Petri dish,
- Close the Petri dish,
- Reflame the glass elbow, let it cool, and return it to the alcohol solution,
- Secure the Petri dish with several pieces of tape and
- Place upside-down (agar hanging) Petri dish in incubator.

Next session you will look at the growth of colonies on the surface of the plate to see if your sample was contaminated.

**EXERCISE #2**

Observe the sample plates of *Escherichia coli*, *Serratia marcescens* and *Micrococcus luteus*. Compare the size, shape, height, color, and other features of their colonies and record your observations. Remember! Each colony is a group of many hundreds to thousands of individual organisms.

**EXERCISE #3**

Each member of a two-person team needs to obtain another clean, closed Petri dish that contains nutrient agar.

Each team needs to use the same unknown culture that they used for procedure #1. Keep the solution closed until it is time to use it.

Again observe the location of the Bunsen burner on your lab table. Once again use the burner flame to sterilize the opening of your unknown culture tube when you are ready to open it, and before you close it again.

You must also remember to flame the inoculation loop before and after its use.
Be certain to remember to mark the outer bottom cover of the Petri dish (use tape or grease pencil) with your name.

Recall that Petri dishes must be stored upside down (agar hanging) in the incubator, to prevent moisture from washing away the organisms growing on the surface of the nutrient agar.

You will now use an alternative method for preparing a bacterial growth plate: the **streak** method.

- Turn the Petri dish right side up,
- Open the unknown culture, and *flame* the opening,
- Flame the *inoculation loop* and let it cool until the red color disappears,
- Place the loop end of the inoculation wire into the unknown culture,
- Withdraw the inoculation loop,
- Flame the opening of the tube,
- Close the tube,
- Carefully streak the inoculation loop across the agar using the pattern shown below (*NOTE: DO NOT* break the surface of the agar),
- Close the Petri dish,
- Flame the loop,
- Tape the Petri dish shut and
- Place the Petri dish upside down in the incubator.