Aim: Preparation of LB medium and autoclaving.

What is microbiology?
Study of microorganisms (microbes), organisms small in size, which require microscopes to be visualized. However, certain microorganisms, some algae and bacteria, can be seen by naked eye. Thus, additional criteria to enlist an organism as microorganism are:
-organisms that are simple in architecture and not highly differentiated.
-require special techniques to isolate the organism from environment e.g., culture media and sterilization techniques.

Microorganisms encompass all three domains of life (Bacteria, Archaea, Eukarya):
Prokaryotic microorganisms: archaea and bacteria
Eukaryotic microorganisms: fungi, algae, and protozoa

Media for culturing microorganisms:
Media is a combination of biologically and chemically derived compounds that support the growth of microorganisms. Media should provide all elements and ions required for the growth of an organism (e.g., C, H, O, N, S, P, Na, Ca, Mg, Mn, K, etc.). Certain microorganisms require growth factors to be supplied in the media i.e., factors that are essential for the growth of microorganism but the microorganism cannot synthesize them e.g., vitamins, certain amino acids. Thus the type of culture media used depends on the kind of microorganism to be studied since different microorganisms have different nutritional requirements.

Environmental parameters important for growth of microorganisms:
Temperature, pH, salt concentration, pressure, gases.
Thus, to grow a particular microorganism in the laboratory we need to provide appropriate culture media and adequate environmental conditions.

LB media: LB is a very commonly used media in the laboratory. LB is rich in nutrients and thus supports the growth of most microorganisms.
LB stands for Lysogeny Broth. Composition of LB was first given by Giuseppe Bertani in 1951 and was used to isolate phage (a virus that infects bacteria). The composition of LB was later modified by Luria in 1960's, so LB has mistakenly been expanded as 'Luria Bertani' medium or 'Luria Broth' or 'Lennox Broth'.

The common composition of LB is given below. The composition is sometimes modified to reduce salt concentration or to make LB more rich by adding glucose etc.
Tryptone: 10g
Yeast extract: 5g
NaCl: 10g
Tryptone: mixture of peptides, peptones and amino acids formed by the digestion of casein by trypsin. Casamino acids are similar to tryptone and are sometimes used in LB instead of tryptone. Casamino acids are made by acid hydrolysis of casein, and hence have only free amino acids.
Yeast extract: soluble extract of yeast cells
NaCl: salt for maintaining osmotic balance
All the above components are available commercially both as individual components as well as ready-mix where the components have already been mixed in a defined ratio.
LB is a complex (undefined) media since elemental composition of LB is unknown. Simple (defined or synthetic) media are also available where the exact ratio of elements in the media is known.

Laboratory Practical:
Make 100 ml of LB media in 250 ml flask using the LB ready-mix provided in the lab. Measure the pH of media using pH paper. pH should be ~7.2-7.6. Plug the flask with cotton plug and cover the cotton plug with Aluminium foil.

Media Sterilization:
Since we want to grow a microorganism of interest in the media prepared above, the media should be free of contamination. In the laboratory, the equipment used to sterilize media is 'autoclave'. Autoclave was discovered by Chamberland in 1884.

In an autoclave media is sterilized by steam at a temperature of 121 °C and pressure of 15 psi (pounds per square inch) for 15-20 min.

A special tape, called autoclave tape, is used as an indicator of whether the sterilization is complete. Tapes have chemical compound that changes color on sufficient heating in the autoclave.