

## **Aim: Pouring LB Agar plates.**

### Properties required for a solidifying agent

1. Should not be inhibitory to the growth of microorganism.
2. Should not be digested by the microorganism.
3. Should remain solid at the incubation temperature used for the growth of microorganism.

The first solid medium employed by Robert Koch (1843-1910) was an aseptically cut potato slice. The next solidifying agent used by Koch was gelatin, a protein. Gelatin was not a satisfactory agent since it suffered from two problems: 1) melts at 37 °C, favored incubation temperature for many bacteria; and 2) many bacteria can digest gelatin.

### Agar as a solidifying agent

Agar is the most commonly used solidifying agent, and its use as a solidifying agent was introduced by Walter Hesse (a co-worker in Koch's laboratory) in 1882 on suggestion by his wife Fannie Hesse.

Agar is an extract of algae. It is a complex polysaccharide. It is a mixture of two components: the linear polysaccharide agarose, and a heterogeneous mixture of smaller molecules called agarpectin. Agar is the preferred solidifying agent because: 1) it cannot be digested by most microorganisms; 2) it does not inhibit the growth of a majority of microorganisms; and 3) it remains solid at 37 °C. Agar melts at 100 °C and remains liquid down to a temperature of 45 °C.

To prepare solid media, Agar is generally added at a concentration of 1.5-2%. To prepare LB Agar, Agar is added to LB liquid media. The suspension is autoclaved. To prepare solid media plates, when LB Agar cools down to a temperature of ~45 °C, LB Agar is poured in Petri plates.

A co-worker in Koch's laboratory, Richard Petri, developed the plate (in 1887) and the plate was thus named in his honor as Petri plate. The design of the petri plate has remained unchanged from the original design except that now-a-days plastic plates are used instead of glass plates.

### Use of solid versus liquid media

Liquid media is generally used for applications such as preparation of genomic DNA, plasmid DNA, proteins etc. in large amounts. For analysis of growth of microorganism over time liquid media is preferred.

Solid media are used for applications such as determining the viability of microorganisms by colony counting, isolating microorganisms from environment etc.

### Equipments/reagents required to maintain sterilizations conditions during microbiological handling

1. Laminar Hood (HEPA filter, UV light); HEPA: high efficiency particulate air
2. Flame
3. 70% alcohol