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**HLP (Hsu's *Lactobacillus-Pediococcus*) Medium Instructions**

**Derived from Siebel Institute of Technology**

Propose: Used for detection and enumeration of Lactic Acid Bacteria

**Detection of Bacteria with HLP tubes**

1. To use, loosen the screw-caps and place the tubes in a boiling water bath to liquefy medium. When the medium has been liquefied. Close the caps tightly and cool to 40 °C before inoculation.
2. Pipette a 0.1 to 1.0 ml portion of the test sample (or diluted sample) into a cool tube containing HLP.
3. Recap the tube and gently invert twice to distribute any microorganisms contained in the inoculum uniformly throughout the medium.
4. Place the closed tubes in an incubator (an anaerobic environment is NOT required) at 82-86 °F.
5. Examine tubes after 48 hours of incubation for a preliminary count, and after 72 to 96 hours for a final count.
6. If it is suspected that the sample may be heavily contaminated with acetic acid bacteria, 2 to 4 ml of sterile paraffin may be used to overlay the surface of the medium after inoculation in order to suppress the growth of these bacteria.

**Detection of Bacteria with HLP tubes (membrane filtration)**

1. Filter 10 ml to 100 ml of the test sample through a membrane filter (0.45 µm pore size).
2. Transfer the membrane onto the surface of the solidified HLP in the Petri dish.
3. Hold the plates at 82-86 °F and examine after 72 to 96 hours of incubation. (An anaerobic environment *IS* required to encourage the growth of certain lactic acid bacteria)

**Notes:**

*Lactobacillus* are identifiable as light/white inverted tear drop shape colonies, while *Pediococcus* are observed as light/white spherical/round colonies.