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LABORATORY MEDIA

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# HLP Medium

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

**SELECTIVE DETECTION AND ENUMERATION OF *LACTIC ACID (LACTOBACILLUS AND PEDIOCOCCUS) BACTERIA***

Preparation of HLP tubes

1. Suspend 7 grams of HLP in 100 ml of distilled water in a 500 ml Erlenmeyer flask. Close the flask with a cotton plug or other permeable closure.
2. Dissolve the dry medium by bringing the contents of the flask to boiling. If direct fire is used, swirl the flask frequently during heating to avoid sticking or scorching. Boil the medium for two to three minutes.
3. While the medium is still hot, transfer approximately 17 ml to each of 6 sterile screw-cap type tubes (16 x 150 mm).* This should give a depth of medium of about 110 mm. Close screw-caps tightly.
4. a) Freshly filled HLP tubes may be used immediately after cooling to about 40ºC. b) Alternatively, the HLP tubes may be stored a 4ºC to 5ºC for later use. Do not store for longer than 2 weeks. 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 ºC before inoculation.

Detection of Bacteria with HLP tubes

1. Pipette a 0.1 to 1.0 ml portion of the test sample (or diluted sample) into a cool tube containing HLP.
2. Recap the tube and gently invert twice to distribute any microorganisms contained in the inoculum uniformly throughout the medium.
3. Place the closed tubes in an incubator (an anaerobic environment is NOT required) at 28-30ºC.
4. Examine tubes after 48 hours of incubation for a preliminary count, and after 72 to 96 hours for a final count.
5. 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.

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Membrane Filtration

1. Use 7 grams of HLP plus an additional 1.5 to 2.0 grams of agar (obtained separately) per 100 ml distilled water.
2. After mixing, dissolving and boiling HLP and agar mixture, pour 10 to 15 ml into sterile Petri dishes (60 x 15 mm) and allow the medium to solidify at room temperature for about one hour before use. (Plates may be stored at ~ 4ºC for 2 weeks).
3. Filter 10 ml to 100 ml of the test sample through a membrane filter (0.45 µm pore size, 47 mm diameter).
4. Transfer the membrane onto the surface of the solidified HLP in the Petri dish.
5. Hold the plates at 28-30ºC and examine after 72 to 96 hours of incubation. (An anaerobic environment IS required to encourage the growth of certain lactic acid bacteria)

**Notes:**

1. It is not necessary to autoclave this medium before use.
2. *Lactobacillus* are identifiable as light/white inverted tear drop shape colonies, while *Pediococcus* are observed as light/white spherical/round colonies

*Available from most chemical supply companies.