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Use of Peptone Iron Agar for the Detection of Hydrogen Sulfide

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When using Peptone Iron Agar (Difco) to detect hydrogen sulfide production, test organisms are normally stabbed into the medium (as recommended in the Difco Manual). However, this test is sometimes performed with the medium in slope cultures (Turri and Silvestri, Ann. Microbiol. Enzimol. **10**:71, 1960; Küster and Williams, Appl. Microbiol. **12**:46, 1964). Certain discrepancies in the results obtained were noted by these workers and by us. Organisms which blackened the medium in stab culture did not do so in slope or plate culture. Therefore, a series of experiments was performed in an attempt to explain these observations.

Two *Enterobacteriaceae* species (*Proteus mirabilis* and *P. vulgaris*) were used to set up duplicate stab, slope, and plate cultures on Peptone Iron Agar, with lead acetate strips inserted into all cultures. After 2 days of aerobic incubation at 25 C, one set of cultures was incubated for an additional 2 days under hydrogen; the other set was left under aerobic conditions. Observations of the medium and the lead acetate strips were then noted. In all cultures, darkening of the strips occurred, there being no observable difference in its intensity. Blackening of the medium in plate and slope cultures occurred only under anaerobic conditions. The stab cultures blackened to the surface of the medium in anaerobic conditions, but in the tubes incubated aerobically no sulfide was visible in the top few millimeters of the medium.

A parallel set of tests was made with uninoculated medium completely blackened by chemically produced hydrogen sulfide. Similar results were obtained, the sulfide being stable under anaerobic conditions. In aerobic conditions, the blackening disappeared within 12 hr from plates and slopes and gradually disappeared from the top downwards in unsloped tubes. A 0.05% solution of the indicator (ferric ammonium citrate) in agar produced identical results. Thus, the constituents of the medium did not influence this change.

It was evident that the sulfide was unstable in the presence of atmospheric oxygen, and hence the results obtained were due to the chemical behavior of the sulfide rather than to the ability of the organisms to form hydrogen sulfide. Chemical tests on the medium and a 0.05% solution of ferric ammonium citrate indicated that the sulfide formed was oxidized to sulfur and ferric compounds.

Therefore Peptone Iron Agar should not be used in slope cultures for detection of hydrogen sulfide. Plate cultures have the same defect, but clear positive results have been obtained by covering young colonies with sterile cellophane discs or Vaseline (Goodfellow and Gray, *in press*). Stab cultures gave satisfactory results, but the clearest positives, with a more intense and evenly distributed blackening of the medium, were obtained by use of the shake tube method of inoculation.