



Hoyle's Medium

LAB 27

Description

A highly selective culture medium for the isolation and differentiation of *Corynebacterium diphtheriae* types *gravis*, *mitis* and *intermedius*. Hoyle's medium gives rapid growth of all types of *C. diphtheriae*, which results in most specimens giving adequate growth with overnight incubation.

| Formula | g/litre |
|-----------------|---------|
| Beef Extract | 10.0 |
| Peptone | 10.0 |
| Sodium chloride | 5.0 |
| Agar | 12.0 |

Method of Reconstitution

Weigh 37 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C, add 50ml of lysed horse or sheep blood and 10ml of X027 potassium tellurite solution. Mix well before pouring.

Appearance: Dark Red, clear gel.

pH: 7.8 ± 0.2

Inoculation

Spread the entire surface with the swab or sample under investigation. Hoyle's medium is very selective and spreading for single colonies using a wire loop is not necessary. Use of a nonselective blood agar alongside Hoyle's is recommended.

Incubation: 37°C for 18-48 hrs, aerobically

| Interpretation | | | | |
|--|------------------|-----------------|--------------------------|--|
| organism | colony size (mm) | shape & surface | colour | other |
| <i>C. diphtheriae</i> var <i>gravis</i> | 1.5-2.5 | CV.CR.D | Grey(daisy head) | Colonies difficult to emulsify |
| <i>C. diphtheriae</i> var <i>mitis</i> | 0.5-2.0 | CV.E.G. | Grey (dark centre) | Easily emulsified |
| <i>C. diphtheriae</i> var <i>intermedius</i> | 0.5-1.0 | CV.E.G. | Grey (dark centre) | |
| <i>C. Ulcerans</i> | 1.0-1.5 | CV.E.G. | Grey (dark centre) | Streptococcal appearance in Gram stain |
| <i>C. hoffmanii</i> | 0.5-1.0 | CV.E.G. | Black (confluent growth) | White/grey colonies |
| <i>C. xerosis</i> | 0.5-2.5 | CV.E.G. | Black | |
| <i>Streptococcus</i> spp | pp - 1.5 | CV.E.G. | Black | Enterococci may. be larger |
| <i>H. influenzae</i> | pp – 1.5 | CV.E.G. | Grey/black | Some strains no growth |

Storage: Plates – up to 7 days at 2-8°C

Minimum Q.C. Organisms: *C. diphtheriae* var *mitis* (non-toxigenic)
E. coli NCTC 10418 (inhibition)

References

Hoyle L. (1941) A Tellurite Blood Agar Medium for the Rapid Diagnosis of Diphtheria. Lancet 1 175-176
 176. Elek S.D. (1948) The Recognition of Toxigenic Bacterial Strains in vitro. Brit. Med. J. 1 493-496.