

Malachite-green Broth, Base

For the selective enrichment of *Pseudomonas aeruginosa* according to HABS and KIRSCHNER (1943).

The culture medium has been recommended by SCHUBERT and BLUM (1974) for water testing and has been accepted by the Deutsches Institut für Normung (German Institute for Standardization, DIN) in the respective DIN-Standard 38411, Part 8, for the examination of ground-, surface-, drinking-, bathing and process-water. In addition, it is suitable for the examination of mineral and spring-water.

Mode of Action

The malachite-green essentially suppresses growth of the accompanying flora while leaving *Pseudomonas aeruginosa* virtually unaffected. The addition of a small amount of phosphate buffer is favourable for maintaining the correct pH of the broth.

Typical Composition (g/litre)

Peptone from meat 5.0; meat extract 3.0; di-potassium hydrogen phosphate 0.37.

These values are valid for the single-strength broth.

Preparation

Base Broth:

Suspend 8.4 g (single-strength broth) or 25.1 g (triple-strength broth) in 1 litre of demineralized water. Dispense in 50 ml volumes into suitable culture vessels and autoclave (15 min at 121 °C).

The broth base is clear and yellow-brown.

Malachite-green solution:

Suspend 0.15 g malachite-green oxalate in 90 ml of demineralized water and sterilize by filtration.

Complete broth:

Add to 50 ml of cooled base broth 0.3 ml (single-strength broth) or 0.9 ml (triple-strength broth) of the malachite-green solution under sterile conditions.

pH: 7.3 ± 0.2 at 25 °C.

The complete broth is clear and green.

Alternative method of preparation:

If preferred, the malachite-green solution may also be added before distributing the base broth into the vessels. In this case it has to be autoclaved first. Then add to 1 litre of base broth 6 ml (single-strength broth) or 18 ml (triple-strength broth) of malachite-green solution under sterile conditions and dispense in 50 ml volumes into sterile vessels.

Experimental Procedure and Evaluation

An optimal enrichment of *Pseudomonas aeruginosa* requires a concentration of 0.01 g /l malachite-green oxalate. Therefore, small sample-volumes (5 ml or less and solid materials such as membrane filters) will be directly inoculated into 50 ml of single-strength broth. In the case of high sample-volumes, 2 parts of sample are added to 1 part of triple-strength broth (e.g. 100 ml of water to 50 ml of broth). Thus, final concentration of the inoculated broth will always be single-strength.

Incubation: 24 ± 4 to 44 ± 4 hours at 35 °C ± 1 °C.

Cultures showing turbidity, i.e. growth after the incubation are regarded positive. Growth may, but does not have to, be accompanied by a change of colour. Positive cultures are further examined to confirm the presence of *Pseudomonas aeruginosa* according to standard procedures.

Literature

DIN Deutsches Institut für Normung e.V.: Deutsches Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Mikrobiologische Verfahren (Gruppe K). Nachweis von *Pseudomonas aeruginosa* (K 8). – **DIN 38411**.

HABS, H., a. KIRSCHNER, K.H.: Der Pyocyanus-Meerschweinchenhautversuch zur Prüfung von Hautdesinfektionsmitteln. – **Z. Hyg.**, **124**; 557-578 (1943).

SCHUBERT, R., a. BLUM, U.: Zur Frage der Eignung der Malachitgrün-Bouillon nach HABS und KIRSCHNER als Anreicherungsmedium für *Pseudomonas aeruginosa* aus dem Wasser. – **Zbl. Bakt. Hyg., I. Orig. B.**, **158**: 583-587 (1974).

Ordering Information

| Product | Merck Cat. No. | Pack size |
|-----------------------------|----------------|-----------|
| Malachite-green Broth, Base | 1.10329.0500 | 500 g |
| BROLACIN Agar | 1.01638.0500 | 500 g |
| Malachite-green oxalate | 1.01398.0025 | 25 g |

Quality control

| Test strains | Inoculum | Growth on BROLACIN Agar after | |
|---|--------------|-------------------------------|----------|
| | | 20 hours | 44 hours |
| <i>Pseudomonas aeruginosa</i> ATCC27853 | approx. 1 % | ≥ 50 % | ≥ 80 % |
| <i>Enterobacter aerogenes</i> ATCC15038 | approx. 99 % | ≤ 50 % | ≤ 20 % |