

INSTRUCTIONS FOR USE – READY-TO-USE PLATED MEDIA

# BD™ Tellurite Agar (Hoyle)

#### **INTENDED USE**

**BD Tellurite Agar (Hoyle)** is a partially selective and differential medium for the isolation of *Corynebacterium diphtheriae* from clinical specimens.

## PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

**BD Tellurite Agar (Hoyle)** is a modification of the medium developed by Neill for the isolation of *Corynebacterium diphtheriae* that allows growth of all biovars of *C. diphtheriae*.<sup>1,2</sup> Meat Extract and peptone supply nitrogen and growth factors. Sodium chloride maintains the osmotic stability. Potassium tellurite at the concentration chosen inhibits Gram negative and a variety of Gram positive bacteria and allows detection of tellurite reduction which is typically but not exclusively found in corynebacteria.<sup>3</sup> Lysed horse blood provides additional nutrients.

#### REAGENTS

#### **BD Tellurite Agar (Hoyle)**

Approximate Formula\* Per Liter Purified Water

<u>Approximate i officia i ci Elter i di</u>	neu water
Meat Extract	10.0 g
Peptone	10.0
Sodium Chloride	5.0
Potassium Tellurite	0.35
Horse Blood, defibrinated, lysed	7%
Agar	15.0 g
pH 7.8 +/- 0.2	

\*Adjusted and/or supplemented as required to meet performance criteria.

# PRECAUTIONS

IVD . For professional use only.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

Observe special biohazard risks when handling specimens suspected to contain C. diphtheriae.

#### STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

#### USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate for 24 to 48 hours aerobically at 35 to 37° C.

Strains	Growth Results
Corynebacterium diphtheriae ATCC™ 9675	Growth good to excellent; small to medium- sized dark grey to black colonies
Corynebacterium diphtheriae ATCC 11913	Growth good to excellent; medium-sized dark grey to black colonies

Escherichia coli ATCC 25922	Inhibition partial to complete
Staphylococcus aureus ATCC 25923	Inhibition partial to complete
Uninoculated	Red, slightly opaque

# PROCEDURE

## Materials Provided

**BD Tellurite Agar (Hoyle)**, provided in 90 mm Stacker™ plates. Microbiologically controlled.

#### Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

## Specimen Types and Transport

This medium is used for the (partially) selective isolation of *Corynebacterium diphtheriae* from clinical specimens (e.g., swabs from the inflamed areas of the throat and nasopharynx; or swabs from wounds) of patients suspected to be infected with diphtheria (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**). It is recommended to inoculate two swabs with the specimen, one of which is used for culture, and the second one is used for a differential stain and microscopy. Swabs should be transported directly to the laboratory or should be immediately streaked onto BD Tellurite Agar (Hoyle), and the inoculated plate should then be sent to the laboratory. If this is not possible, swabs should be sent to the laboratory in special tubes containing silica gel.<sup>3</sup>

Note that the diagnosis of diphtheria is primarily based on clinical findings. The laboratory must be informed on the presumptive clinical diagnosis before the specimen is processed.<sup>3</sup>

#### Test Procedure

Streak the specimen directly after arrival in the laboratory. If inoculated plates have been shipped, eventually streak for dilution. Also include a non-selective blood agar such as **BD Columbia Agar with 5% Sheep Blood** and one **BD Columbia CNA Agar with 5% Sheep Blood** plate. Incubate aerobically or in an aerobic atmosphere enriched with carbon dioxide at 35 to 37° C for 24 to 72 hours, and read after 24, 48, and 72 hours.

A differential stain such as Neisser stain must be performed either from the specimen or from Loeffler Serum slants, which are inoculated with the specimen or with a culture of the isolated strain.

# Results

On **BD Tellurite Agar (Hoyle)**, *Corynebacterium diphtheriae* produces gray to black colonies on the medium. Colonies may be inspected with a magnifying glass for determination of the biovars:

*C. diphtheriae* biovar *gravis*: Gray colonies with a matt surface; slightly convex with lobate borders.

*C. diphtheriae* biovar *mitis*: Gray colonies with shiny surface and even borders, often different colony sizes are observed from one culture.

*C. diphtheriae* biovar *intermedius*: Gray colonies with shiny surface and dark gray to black center; relatively small.

*C. hofmannii*: Isolated colonies white to grayish, may appear black in areas of confluent growth. *C. xerosis*: Shiny black colonies.

Streptococci: Tiny black or brownish colonies.

In the Neisser differential stain, coryneform rods containing metachromic granules may be seen if *C. diphtheriae* is present. Further biochemical tests are necessary for confirmation of the species. Appropriate tests must be applied to all isolates to verify toxin production. For a complete discussion on procedures for diagnosis of diphtheria, refer to the references.<sup>3,4</sup>

# PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

**BD Tellurite Agar (Hoyle)** is one of the media recommended for the isolation of *Corynebacterium diphtheriae* from clinical specimens.<sup>2</sup>

Diagnosis of diphtheria requires multiple tests and media since other organisms may mimick the colony appearance of *C. diphtheriae* and its biovars. The diagnosis must not be only based on typical growth on this medium. Immunological procedures for the detection of diphtheria toxin from the isolates must be performed to confirm the diagnosis. Consult the appropriate references<sup>3,4</sup>

**BD Tellurite Agar (Hoyle)** is not completely selective for *C. diphtheriae*. Other corynebacteria and other bacterial species may grow on this medium.

## REFERENCES

- 1. Hoyle, L. 1941. A tellurite blood agar medium for the rapid diagnosis of diphtheria. Lancet i: 175-176.
- 2. MacFaddin, J.F. 1985. Media for the isolation cultivation maintenance of medical bacteria. Volume 1. Williams and Wilkins, Baltimore, London
- 3. Funke, G., and K.A. Bernard.2003. Coryneform gram-positive rods. *In:* Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
- Baron, E. J., L. R. Peterson, and S. M. Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

#### PACKAGING/AVAILABILITY

**BD Tellurite Agar (Hoyle)**Cat. No. 256044Ready-to-use Plated Media, cpu 20

#### FURTHER INFORMATION

For further information please contact your local BD representative.

# -

#### Becton Dickinson GmbH

Tullastrasse 8 – 12 D-69126 Heidelberg/Germany Phone: +49-62 21-30 50 Fax: +49-62 21-30 52 16 Reception\_Germany@europe.bd.com

http://www.bd.com/europe/regulatory/

ATCC is a trademark of the American Type Culture Collection BD and BD logo are trademarks of Becton, Dickinson and Company. © 2011 BD