BD™ Helicobacter Agar, Modified

INTENDED USE

BD Helicobacter Agar, Modified is a selective medium for the isolation of *Helicobacter pylori* from gastric specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Since its first isolation in1982 by Marshall and Warren, *Helicobacter pylori* has been shown to be an important infectious agent, responsible for chronic gastritis, duodenal, peptic ulcers and certain types of stomach cancer.[1,2] Although serological tests for the presence of antibodies against the organism or rapid urease tests, detecting the unusually active urease of the organism, are frequently applied for diagnosis, culture is needed to detect an early infection when an antibody response might be still be absent. Furthermore, culture is needed to determine the antimicrobial susceptibility pattern of individual strains. Several media have been used for the isolation of the organism which is not extremely fastidious, but very sensitive to oxygen, since it is a microaerophile, and requires an incubation period of 3 to 5 days.[3]

BD Helicobacter Agar, Modified contains Columbia Agar as a base. The antimicrobial combination is the formulation described by Dent and McNulty, which contains combinations of vancomycin, amphotericin B, trimethoprim and cefsulodin to inhibit contaminating flora without loss of recovery of *H. pylori.*[4] As proposed by Stevenson and colleagues, the cefsulodin concentration has been increased to provide improved inhibition of contaminating flora.[5] Lysed horse blood is added to provide additional nutrients.

REAGENTS

BD Helicobacter Agar, Modified

Formula* Per Liter Purified Water

Pancreatic Digest of Casein	12.0 g	Agar	13.5 g
Peptic Digest of Animal Tissue	5.0	Vancomycin	0.01
Yeast Extract	3.0	Amphotericin B	0.005
Beef Extract	3.0	Trimethoprim	0.02
Corn Starch	1.0	Cefsulodin	0.01
Sodium Chloride	5.0	Horse Blood, lysed	7%

pH 7,3 +/- 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.

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 at 35 to 37° C in a microaerobic atmosphere, e.g. in a **BD GasPak™** jar with an atmosphere provided by using the **BD CampyPak™** system (including the catalyst) or the **BD CampyPak Plus** system for 3 to 5 days.

Strains	Growth Results
Helicobacter pylori ATCC™ 43504	Growth good to excellent; tiny to medium-sized, transparent colonies
Candida albicans ATCC 10231	Inhibition partial to complete
Escherichia coli ATCC 25922	Inhibition partial to complete
Proteus mirabilis ATCC 43071	Inhibition partial to complete; swarming inhibited
Staphylococcus aureus ATCC 29213	Inhibition complete
Uninoculated	Burgundy red, slightly transparent

PROCEDURE

Materials Provided

BD Helicobacter Agar, Modified (90 mm **Stacker™** plates). Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types, Collection and Transport

Collect several fresh gastric biopsy specimens from the patient, at least one from the gastric antrum and one from the corpus, in a suitable transport medium. Gastric juice is not a suitable specimen. If the specimen can be transported and processed without delay, physiological saline may be used. If a delay is expected, transport media such as Stuart's medium or **BD Port-A-Cul™** must be used and should be held at 4 to 8° C, for not longer than 24 h before processing. The organism is extremely sensitive to desiccation and exposure to oxygen.[6] It has been shown that glycerol added to transport media improves viability if kept refrigerated (e.g., at +4° C) or frozen.[7]

Test Procedure

The agar surface should be smooth and moist, but without excessive moisture. Plates showing signs of desiccation, such as shrinkage of the medium, must not be used.
During handling of the specimens and cultures of the organism avoid prolonged exposure to air because the organism is very oxygen sensitive.[6]
Biopsy specimens should be grinded or minced with a small amount of sterile physiological saline before they are applied to the medium. The homogenate should be placed immediately on the medium surface and should be caught with the loop and then streaked over the surface using an isolation streak method. A non-selective medium such as **BD Columbia Agar with 5% Horse Blood** or **BD Chocolate Agar (GC Agar with IsoVitaleX)** should be inoculated together with **BD Helicobacter Agar, Modified**, to obtain a full recovery of the pathogens involved.
Incubate the inoculated plates for 3 to 5 days at 35 ± 2° C in a microaerobic atmosphere, e.g. in a **BD GasPak** jar with an atmosphere provided by using the **BD CampyPak** system (including a catalyst) or the **BD CampyPak Plus** system.

Results

After incubation, the plates should show isolated colonies in the areas where the inoculum was appropriately diluted. *Helicobacter pylori* colonies are tiny to medium-sized and transparent.
A Gram stain from respective colonies will reveal Gram negative, slightly curved rods. A positive rapid urease, oxidase, and catalase reaction which can be directly performed with growth from the isolation plate (if sufficient growth is available) are indicative of *H. pylori*. Final identification should be done using appropriate biochemical tests.[6] During handling of the culture, avoid time delays since most *Heliobacter pylori* strains will not survive an exposure to air for longer than 30 to 45 minutes.[6] Subcultures on appropriate non-selective media (see **Test Procedure**) should be made immediately and incubated as described above.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD Helicobacter Agar, Modified is used for the isolation of *Helicobacter pylori* from human gastric specimens. [5,6]

Bacteria other than *Helicobacter pylori* may grow on this medium. This can include *Helicobacter* species other than *H. pylori* or contaminants from normal flora.

Growth from this medium must be further differentiated using biochemical, morphological or molecular tests. Stool specimens should not be applied to **BD Helicobacter Agar, Modified**, since the medium may not be sufficiently selective to suppress intestinal flora.

This medium has not been tested for growth of *Helicobacter* species other than *H. pylori*.

REFERENCES

1. Warren, J.R., and B.J. Marshall. 1983. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet i: 1273-1275.
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3. Goodwin, C.S., and J.A. Armstrong. 1990. Microbiological aspects of *Helicobacter pylori* (*Campylobacter pylori*). Eur. J. Clin. Microbiol. Infect. Dis. 9: 1-13.
4. Dent, J.C., and C.A.M. McNulty. 1988. Evaluation of a new selective medium for *Campylobacter pylori*. Eur. J. Clin. Microbiol. Infect. Dis. 7: 555-568.
5. Stevenson, T.H., L.M. Lucia, and G.R. Acuff. 2000. Development of a selective medium for isolation of *Helicobacter pylori* from cattle and beef samples. Appl. Environ. Microbiol. 66: 723-727.
6. Jerris, R.C. 1995. *Helicobacter. In:* Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
7. Han, S.W., et al. 1995. Transport and storage of *Helicobacter pylori* from gastric mucosal biopsies and clinical isolates. Eur. J. Clin. Microbiol. Infect. Dis. 14: 349-352.

PACKAGING/AVAILABILITY

BD Helicobacter Agar, Modified

Cat. No. 254430 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

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