C-H-O MEDIUM w/ and w/o CARBOHYDRATE

INTENDED USE
Remel C-H-O Medium w/ and w/o Carbohydrate is a liquid medium recommended for use in qualitative procedures for the determination of fermentation reactions of anaerobic bacteria.

SUMMARY AND EXPLANATION
Carbohydrate utilization patterns play an important role in the identification of anaerobes.[1] Anaerobes, because of a less efficient metabolism, require carbohydrate utilization media to be richer in auxiliary growth factors and to contain a higher concentration of peptone as well as carbohydrate. C-H-O Medium meets the requirements of even fastidious anaerobes. C-H-O medium is listed in the CDC Laboratory Manual of *Laboratory Methods in Anaerobic Bacteriology* as a fermentation base for anaerobic bacteria.[2,3]

PRINCIPLE
Casein peptone, yeast extract, and L-cystine supply essential nutrients for the growth of bacteria. Sodium thioglycollate is a reducing agent, which lowers the oxidation-reduction potential of the medium and, thereby, creates and maintains an anaerobic environment. The indicator, brom thymol blue, changes from green to yellow as a result of the production of acid from the utilization of a specific carbohydrate. This indicator change occurs at a pH of 6.0 or below.

REAGENTS (CLASSICAL FORMULAE)*
Base Medium:

Casein Peptone	15.0	g
Yeast Extract	7.0	g
Sodium Chloride	2.5	g
Sodium Thioglycollate	0.5	g
L-Cystine	0.25	g
Ascorbic Acid	0.1	g
Brom Thymol Blue	0.01	g
Agar	0.75	g
Demineralized Water	1000.0	ml

pH 7.0 ± 0.2 @ 25°C

These optional ingredients are available per liter of medium:

Arabinose	6.0	g
Cellobiose	6.0	g
Dextrose	6.0	g
Fructose	6.0	g
Galactose	6.0	g
Lactose	6.0	g
Maltose	6.0	g
Mannitol	6.0	g
Mannose	6.0	g
Raffinose	6.0	g
Rhamnose	6.0	g
Salicin	6.0	g
Sucrose	6.0	g
Trehalose	6.0	g
Xylose	6.0	g

*Adjusted as required to meet performance standards.

PROCEDURE
1. The inoculum must be from a young, actively growing broth culture (without carbohydrate) of at least 2+ turbidity. Inoculate C-H-O Medium near the bottom of the tube with a sterile pipette.
2. Expel the air from the pipette before placing it in the medium.
3. C-H-O Medium Base Control should be inoculated and incubated in parallel with the fermentation test as a control.
4. Incubate anaerobically, with caps loosened, at 33-37°C for up to 7 days.
5. Acid production as an indication of fermentation is determined by inspecting the tubes on days 1, 2, and 7. The brom thymol blue indicator in the medium will turn yellow at pH 6.0 or lower (acid). (**Note:** Some clostridia may reduce the indicator giving the appearance of a negative reaction. To confirm a negative, transfer 2-3 drops from each tube to a separate tube or spot plate and add 2-3 drops of 1% Brom Thymol Blue Reagent (REF R21203). Record as acid or negative.)
6. Tubes with an acid reaction (positive) can be discarded. Negative tubes should be reincubated.
7. A final reading should be made at 7 days at which time negatives are confirmed by adding 2-3 drops of Brom Thymol Blue Reagent directly to each C-H-O tube. Record as acid or negative.

INTERPRETATION OF THE TEST
Positive Test - Yellow color development
Negative Test - Blue or blue-green color

QUALITY CONTROL
All lot numbers of C-H-O medium w/ and w/o Carbohydrate have been tested for performance and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. Control organisms should be selected that demonstrate a positive and negative reaction for each carbohydrate tested. If aberrant quality control results are noted, patient results should not be reported.

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BIBLIOGRAPHY

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2. Dowell, V.R. and T.M. Hawkins. 1974. Laboratory Methods in Anaerobic Bacteriology, CDC Laboratory Manual. U.S. Dept. of H.H.S., CDC, Atlanta, GA.
3. Dowell, V.R., Jr., G.L. Lombard, F.S. Thompson, and A.Y. Armfield. 1977. Media for Isolation, Characterization, and Identification of Obligately Anaerobic Bacteria. U.S. Dept. of H.H.S., CDC, Atlanta, GA.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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