Designation: B-78
Deposited Name: Treponema hyodysenteriae Harris et al.
Product Description: Type strain.

Medium
ATCC® Medium 1827: BHI with heat-inactivated fetal bovine serum and glucose
ATCC® Medium 260: Trypticase soy agar/broth with defibrinated sheep blood

Growth Conditions
Temperature: 37°C
Atmosphere: Anaerobic gas mixture, 80% N₂·10% CO₂·10% H₂

Propagation Procedure
1. Thaw vial at room temperature under anaerobic conditions. Aseptically withdraw contents of vial and transfer into a tube of #1827 broth (5 to 6 mL).
2. Transfer 0.5 mL of the suspension to #1827 slants, making a biphasic culture. Inoculate two sheep blood agar plates.
3. Incubate the tubes under anaerobic atmosphere at 37°C for 4 to 6 days. One of the sheep blood agar plates is incubated under anaerobic conditions and the second plate is incubated aerobically to check for purity.
4. Growth can be observed as light turbidity in the broth and as a thin film on the slants and #260 plate. Very strong β-hemolysis is exhibited on the anaerobic blood agar plate. Viable growth can also be observed by looking for active motile helical cells when examining a wet mount of a drop from the broth portion of the biphasic slant under phase contrast. The aerobic plate should show no signs of growth.

ANAEROBIC CONDITIONS:
Anaerobic conditions for transfer may be obtained by either of the following:
- Use of an anaerobic gas chamber, or
- Placement of test tubes under a gassing cannula system hooked to anaerobic gas.

Anaerobic conditions for incubation may be obtained by any of the following:
- Loose screw caps on test tubes in anaerobic chamber,
- Loose screw caps on test tubes in an activated anaerobic gas pack jar, or
- Use of sterile butyl rubber stoppers on test tubes so that an anaerobic gas headspace is retained.

Notes
Anaerobe Systems Brucella Blood plates (AS-111 or AS-141) can be used to analyze colony morphology and purity. Colonies on Brucella agar are a thin layer of growth with entire, glistening, circular, smooth, and flat.

This strain was originally deposited as Treponema hyodysenteriae, and it is used for antimicrobial sensitivity testing (Kitai et al. Chemother. 31: 1935-1938, 1987).

Best growth is obtained with biphasic slants. Growth may be poor in broth. Also, additional incubation time may be required upon initial opening. Subsequent transfers after growth will occur in 48 to 72 hours.

Always use freshly prepared pre-reduced media or pre-reduced media that has been previously prepared but stored under anaerobic conditions. Resazurin in the media is a color indicator for anaerobic conditions. Observance of pink color in medium before use or during incubation shows anaerobic conditions have not been met and oxidation has occurred. Medium should be discarded.

Additional information on this culture is available on the ATCC® web site at www.atcc.org.
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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