Designation: 2011 [ATCC 49313, NADC 1893]
Deposited Name: Flexispira rappini Bryner et al.

Medium
ATCC® Medium 260: Trypticase soy agar/broth with defibrinated sheep blood

Growth Conditions
Temperature: 37.0°C
Atmosphere: Microaerophilic

Propagation Procedure
1. Open vial according to enclosed instructions. Rehydrate contents of vial with 0.5 ml of #18 broth.
2. To obtain a biphasic culture, add 0.4 ml of the suspension to a #260 slant. Add remaining 0.1 ml of the suspension to a #260 plate and streak for isolation.
3. Incubate at 37°C under microaerophilic conditions using an anaerobe jar with an active catalyst and a microaerophilic gas generator pack, or other acceptable method, to obtain microaerophilic conditions. Incubate slant with cap loose.

This is a slow growing organism that requires moist conditions for best growth. Growth at the broth/agar interface of the biphasic slant should occur within three to five days, but little turbidity will be seen. To observe growth, examine a wet mount of the broth under phase microscopy. The organism is a medium size, fusiform shaped, motile bacillus.

Individual colonies will not be observed. Growth appears as a film on the surface of fresh, moist plates, extending out from original inoculation site to fill entire plate, usually within 48 to 72 hours.

The cells do not Gram stain well using traditional procedures. For best results, use a basic fuchsin counterstain in place of the safranin.

Once good growth is obtained, transfer or freeze the culture. Adding an equal amount of 20% sterile glycerol to pooled broth from several biphasic slants followed by freezing in liquid nitrogen or "ultra low temperature" freezer is recommended.

Additional information on this culture is available on the ATCC® web site at www.atcc.org.

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 2

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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