Serial Dilution Instructions (API)
FOR DETECTION OF SULFATE REDUCING BACTERIA

- Product Description:

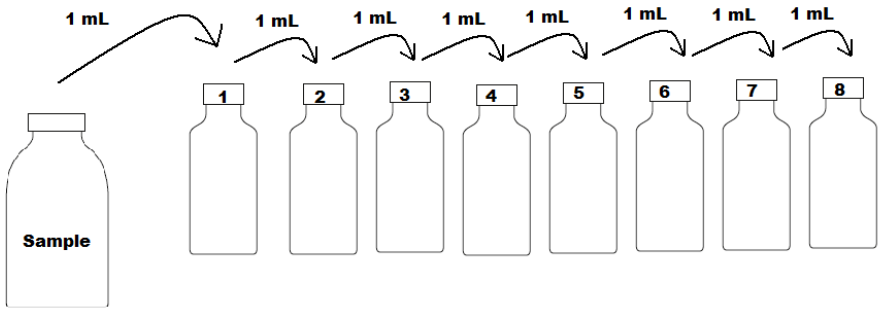
1. N.A.C.E. standard (TM0194-2004) Anaerobic API-RP38
2. Anaerobic bacterial growth media, such as API, is extremely sensitive to oxygen exposure. Biotechnology Solutions' anaerobic bacterial growth media is manufactured inside custom built anaerobic chambers to ensure the highest quality bacterial growth media products.
3. **Physical Characteristics**: This media is clear in color and contains an iron nail.
4. **Chemistry**: API media contains an iron nail in addition to dissolved iron to detect the presence of sulfide. API media contains a carbon and sulfate source as well as other minerals to mimic an environment conducive to SRB growth.
5. **Detection**: Used for the enumeration of Sulfate Reducing Bacteria(SRB) in oil and gas systems, seawater, sediment, or water rich in decaying organic material.

- Collecting a Sample:

1. Collect the sample in such a manner as to preclude contamination from external sources.
2. Time, date, temperature, and appearance of the sample should be recorded.

- Preparation:

1. Arrange selected media vials into "Dilution Series".
2. The selected media should approximate the conditions (Temp., TDS, etc.) of the sample water being tested to avoid the "shock" effect on the microbes.



For additional information, please contact us at:
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- Sterilization:

    1. Wipe the rubber caps of the media vials with sterile alcohol pads.

- Inoculation:

    1. Using a sterile disposable syringe, withdraw 1 mL of the sample and inject it into bottle #1 and discard syringe. Mix contents thoroughly by vigorously agitating the vial. Some bubbles may appear; this is normal.
    2. Make sure to note changes in the vial upon inoculation that may cause a **False Positive**.
        - **False Positive**: If the sample fluid injected into the vial contains a significant concentration of dissolved sulfide, the first one to two vials in the dilution series may turn black. Vials should be recorded as false positives if these vials immediately develop a black precipitate upon inoculation. A vial is NOT considered positive if the nail turns black with no black precipitate present. This is simply an indication of sulfide in the water. If you are not sure if the vial in question is positive for growth or simply a false positive, you can subculture the vial by performing a serial dilution using 1 mL from that vial.
        - We recommend having a water analysis done so that you know what constituents and at what concentration the sample is made of.
    3. With a new sterile syringe, withdraw 1 mL of solution from bottle #1 and inject it into bottle #2 and discard syringe. Mix contents thoroughly by vigorously agitating the vial.
    4. Repeat this process for all the remaining dilution vials (#3 - #6).
    5. Incubate the vials at the temperature at which the original sample was collected (± 2°C). N.A.C.E. standards call for 28-day incubation for SRB media and 14 days for APB media.

- Reading:

    1. **Indicator for (SRB)**: As Sulfate Reducing Bacteria metabolize they reduce sulfate to sulfide. This sulfide then binds with the dissolved iron in the SRB media creating a black precipitate (Iron Sulfide). This black precipitate indicates that the vial is positive for SRB growth.
    2. Record the number of positive vials.

- Disposal:

How should we dispose of Biotechnology Solutions' microbiological media waste?

All of BTS bacterial growth media products are considered to be non-hazardous materials. Media vials may be discarded according to your local, state and federal regulations. To find out more about these regulations please refer to the environmental, health and safety staff at your facility.

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