AMBULATORY CARE NETWORK

COUNTY OF LOS ANGELES   -   DEPARTMENT OF HEALTH SERVICES

Clinical Laboratory Department
POLICY AND PROCEDURE

POLICY NUMBER: 1182
VERSION: 3

SUBJECT: Granada Medium

Purpose:

Granada Medium is used for the primary isolation and detection of beta-hemolytic group B Streptococci from clinical specimens.

Lancefield group B Streptococci (Streptococcus agalactiae), which can be a pathogen in adults, is a leading cause of perinatal and infant mortality. Production of an orange carotenoid pigment on this media is unique to the beta-hemolytic group B Streptococci isolated from humans. Granada Medium, as a primary isolation medium, can detect the pigmented strains, making further subcultures unnecessary.

Granada Medium contains proteose peptone, starch, and horse serum which provide nutrients necessary for growth of bacteria. The medium contains MOPS and sodium phosphate as buffers. Methotrexate enhances the pigment production of group B Streptococci. Dextrose and sodium pyruvate prevent the formation of inhibitory compounds. The selective agents present in the media are metronidazole (to inhibit gram-negative anaerobes), colistin sulfate (gram-negatives), and crystal violet (gram positives).

Storage:

Upon receipt, store media at 2-8°C away from direct light. Media should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is extremely light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

Procedure:

Collect vaginal or rectal or other suspect specimen following the appropriate guidelines for sample collection in Amies, Stuart's or other appropriate transport media without delay.

Streak a plate of Granada Medium with the swab or swabs used to collect the sample. Incubate the inoculated Granada Medium plate in an anaerobic atmosphere for 18-24 hours at 35°C.

Examine plates for orange to red pigmented colonies typical of group B Streptococci. Proceed with appropriate streptococcal tests (gram stain, catalase, and latex agglutination grouping tests) for identification.

Interpretation of Results:

On Granada Medium, colonies of beta-hemolytic group B Streptococci appear red to orange in color. The pigment makes the colonies readily distinguishable from other organisms that may be growing on the plate.

Limitations:

It is recommended that biochemical and/or serological tests be performed on colonies from pure culture for complete identification.

Granada Medium is 88-90% sensitive. Some isolates may be missed if Granada Medium is the only medium used for detection of group B Streptococci.

For pigment development, Granada Medium must be incubated anaerobically. Plates incubated aerobically may yield false negative results.

Non-hemolytic group B streptococci are infrequently (1-2%) found in clinical specimens. These strains do not produce pigment and should be identified by other techniques, e.g. antigen detection or CAMP test.

Granada Medium is not completely selective for S. agalactiae. Other bacteria can grow, mainly from rectal specimens, e.g. enterococci, but do not produce orange or red colonies after 18-48 hours.

Quality Control:

Check for signs of contamination and deterioration. Quality control is performed upon receipt of a new lot or shipment of product. QC should be done with the following organisms: Streptococcus agalactiae ATCC 12386 (growth, orange pigment), Streptococcus pyogenes ATCC 19615 (no pigment), E.coli ATCC 25922 (inhibition) and Bacteroides fragilis ATCC 25285 (inhibition). Refer to the Bacteriology Media QC sheet for complete instructions/form for performing QC.

References:

Hardy Diagnostics, HUGO information sheet, 2002.

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