GARDNERELLA AGAR

PRODUCT:

Plate Media:

Gardnerella Agar, item no. P1675

PURPOSE:

This medium is a selective, differential medium used for the isolation and presumptive identification of *Gardnerella vaginalis* from clinical specimens.

PRINCIPLE:

In 1982 Totten et al.[4] described a selective and differential medium for the isolation of *G. vaginalis*. The medium consists of a basal medium containing the antimicrobics colistin, nalidixic acid, and amphotericin B, and proteose micro peptone and polysorbate 80 for enrichment. A second layer composed of the same ingredients plus 5% human blood is then added. Ninety-five percent of *G. vaginalis* strains produce a characteristic beta hemolysis around the colonies, allowing it to be distinguished from other flora. The antimicrobics contained in the medium inhibit the growth of many other organisms found in conjunction with *G. vaginalis*.[2] *G. vaginalis* shows the greatest degree of hemolysis in shallow layers; therefore, the layer containing blood is an overlay on top of the basal medium to prevent drying and allow for a greater shelf life of the medium.

FORMULA:

Approximate, per liter deionized filtered water.

Columbia CNA Agar	43.0 g
Proteose Micro Peptone	10.0
Amphotericin B	2.0 mg
Polysorbate 80 (7.5% Solution)	1.0 ml
Human Blood (top layer only)	50.0

Final pH 7.3 ± 0.2 at 25°C

PRECAUTIONS:*

For in vitro diagnostic use. Observe approved biohazard precautions.

NOTE: This product contains human blood that has been tested by the American Red Cross and found negative for the presence of hepatitis and AIDS viruses. However, since these tests are inconclusive, proper care should be used in the handling and disposal of this product.

Storage: Upon receipt, store at 2-8°C in the dark. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed.

Limitations: The increased sensitivity of this media allows for the detection of *G. vaginalis* in a high percentage of normal women. Concentrations of the organisms appear to be higher in those women with nonspecific vaginitis. The isolation of *G. vaginalis* in 3-4+ quantities was predictive of clinical findings of nonspecific vaginitis in only 49% of the cases studied. However, a negative predictive value for a negative culture for a patient not having clinical manifestations of the disease was 97%.[1]

This medium is extremely sensitive to exposure to light. Premature lysis of the red cells may occur if the plates are not properly stored.

PROCEDURE:*

Specimen Collection: Information on specimen collection and transport is found in standard reference material on the subject. In general, specimens should be protected from extremes of heat and cold and should be delivered to the laboratory without delay. If there is a delay, suitable transport media such as Amies must be used to maintain the viability of the organisms.

Method of Use: Prior to inoculation, the medium should be brought to room temperature. Roll the specimen swab across the center of the medium. Streak through the inoculated area at a right angle covering the entire surface of the medium. Incubate the inoculated medium at 35°C in 5-10% CO_2 for 48-72 hours.

Interpretation: After incubation of the inoculated plates, examine the surface of the medium for the presence of small, transparent to translucent, hemolytic colonies. If a medium containing sheep blood was inoculated also, *G. vaginalis* would appear as small to pinpoint colonies, but would not produce any hemolysis.[2] *Gardnerella* does not grow on Martin-Lewis or Thayer-Martin media. Perform a Gram stain on any characteristic colonies. Typically, *Gardnerella* will stain as small, gram-variable rods. Cells of a young culture (8-12 hours) may appear gram-positive; in older cultures the organism may appear gram-negative. Colonies on this medium may be used for oxidase and catalase tests, which typically are negative, and the hippurate test, which should be positive. If more definitive identification is preferred, refer to Table 1.

TABLE 1 - CHARACTERISTICS OF *G. VAGINALIS*

Characteristic	Result
Hemolysis of:	
Human red cells	(+)
Sheep red cells	(-)
Hydrolysis of:	
Esculin	(-)
Hippurate	(+)
Starch	(+)
Catalase	(-)
Oxidase	(-)
ONPG	(+)

Material Required but Not Provided: Standard microbiological supplies and equipment such as loops, needles, incubator, and incinerator are not provided.

QUALITY CONTROL:*

Microorganisms Used (ATCC #):	**Expected Results:**
Gardnerella vaginalis (49145)	Growth, beta-hemolysis
Staphylococcus aureus (25923)	Growth
Proteus mirabilis (12453)	Inhibition
Candida albicans (10231)	Inhibition, partial to complete
	Key: See “Interpretation”

User Quality Control: Check for signs of contamination and deterioration.

BIBLIOGRAPHY:

1. Blackman, U., and M. J. Pickett, “Haemophilus vaginalis,” *Unusual Aerobic Bacilli in Clinical Bacteriology*, Scientific Developments Press, Los Angeles, 1978, pp. 41, 42.
2. Greenwood, J. R., “Gardnerella vaginalis,” *Clin. Microbiol. Newsletter*, 3:23-25, 1981.
3. Lennette, E. H., et al., *Manual for Clinical Microbiology*, 4th ed., American Society for Microbiology, Washington D. C., 1985.
4. Totten, P. A., et al., *J. Clin. Microbiol*., 15:141-147, 1982.

*For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

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