Product Insert

ENRICHED TRYPTIC SOY AGAR WITH NALIDIXIC ACID AND VANCOMYCIN (ETSA-NV)

Products

AS-549 Enriched Tryptic Soy Agar with Nalidixic Acid and Vancomycin (ETSA-NV) 4 plates / pkg

Intended Use

Enriched Tryptic Soy Agar with Nalidixic Acid and Vancomycin (ETSA-NV) is an enriched, selective, and differential medium intended for the cultivation and isolation of most gram-negative anaerobic and other fastidious bacteria.

Summary

ETSA-NV is an enriched, selective, and differential medium. The nutritive base consists of casein, soy peptone, yeast extract, and dextrose. The media is supplemented with laked sheep blood and sheep serum to facilitate the recovery, and promote the black pigmentation of, *Prevotella* and *Porphyromonas spp*. Sodium formate and sodium fumarate were included as energy sources for microorganisms that require them for growth. Hemin and vitamin K_1 is added for the enhanced recovery of anaerobic bacteria. The selective agents, nalidixic acid and vancomycin, were added to inhibit most gram-positive anaerobes. This medium is prepared, dispensed, and packaged under oxygen-free conditions to prevent the formation of oxidized products prior to use.

Formulation*

Pancreatic Digest of Casein	15.00	g
Agar	15.00	g
Soy Peptone	5.00	g
Sodium Chloride	5.00	g
Yeast Extract	1.00	g
Dextrose	1.00	g
Sodium Fumarate	1.00	g
Sodium Lactate	0.60	g
Sodium Succinate	0.50	G
Sodium Formate	0.50	g
Potassium Nitrate	0.50	g
Sodium Carbonate	0.40	g
L-Cysteine Hydrochloride (25.0% solution)	2.00	mL
Menadione	1.00	mg
Hemin (0.1% solution)	1.00	mL
Laked Sheep Blood	30.00	mL
Sheep Serum	40.00	mL
Nalidixic Acid	10.00	mg
Vancomycin	2.50	mg
DI Water	1.00	L

Final pH: 7.3 ± 0.3 at 25° C
Final weight: 16.0 g ± 1.6 g

*Approximate formula. Adjusted and/or supplemented as required to meet performance criteria.

Precautions

For *IN VITRO DIAGNOSTIC USE* only. Utilize approved biohazard precautions and aseptic technique when using this product. This product is for use only by properly-trained and qualified personnel. Sterilize all biohazard waste prior to disposal.

Storage and Shelf Life

Storage: Upon receipt, store at room temperature in original package until used. Avoid overheating or freezing. Do not use media if there are signs of deterioration (shrinking, cracking, or discoloration due to oxidation of media) or contamination. The expiration date applies to the product in its original packaging and stored as directed. Do not use product past the expiration date shown on the label.

Shelf Life: 90 days from date of manufacture.

Procedure

Specimen Collection: Protect specimens for anaerobic culture from oxygen during collection, transportation, and processing. Consult appropriate references for detailed instructions concerning collection and transportation of anaerobes.

Methods for Use: ETSA-NV agar should be inoculated directly with clinical specimen or from a broth that has been inoculated with a clinical specimen. Streak plates with inoculum to obtain isolated colonies and immediately place into an anaerobic atmosphere, incubating at 35-37°C for 18-48 hours. Extended periods of incubation may be required to recover some anaerobes. Extended incubation time may also result in loss of selectivity of the media which can result in the overgrowth of organisms that should be inhibited. Detailed instructions for processing anaerobic cultures can be found in the listed references.

Materials Required, But Not Provided

Standard microbiological supplies and equipment such as loops, saline blanks, slides, staining supplies, microscope, incinerator / autoclave, incubators, anaerobic chamber / anaerobic jars, disinfectant, other culture media, and serological / biochemical reagents.

Interpretation of Results

This media supports good growth of many gram-negative anaerobes, along with other fastidious bacteria, isolated from clinical specimens. It also facilitates the pigmentation of the black pigmenting *Prevotella* and *Porphyromonas spp.*

Limitations

ETSA-NV will not provide complete information for identification of bacterial isolates. Additional test procedures and media are required for complete identification. It is recommended that a non-selective media, such as Brucella Blood Agar (BRU, catalog #: AS-111) also be inoculated from the same clinical specimen to assure recovery of all species present. Consult reference materials for additional information.

Quality Control

The following organisms are routinely used for quality control testing at Anaerobe Systems.

Organism Tested	ATCC #	Results	Time	Special Reaction
Bacteroides fragilis	25285	Growth	24 – 48 hrs	
Prevotella melaninogenica	25845	Growth	24 – 48 hrs	Pigment*
Fusobacterium necrophorum	25286	Growth	24 hrs	
Fusobacterium nucleatum	25586	Growth	24 – 48 hrs	
Clostridium perfringens	13124	No Growth		
Peptostreptococcus anaerobius	27337	No Growth		
Prevotella intermedia	25611	Growth	24 hrs	Pigment*
Propionibacterium acnes	6919	No Growth		
Eggerthella lenta	43055	No Growth		
Actinomyces viscosus	43146	No Growth		
Porphyromonas gingivalis	33277	Inhibit to No Growth		

*Pigment production may require more than 48 hours of incubation.

User Quality Control: The final determination to the extent and quantity of user laboratory quality control must be determined by the end user.

If sterility testing is to be performed on this product, a representative sample of the lot(s) should be incubated anaerobically and aerobically for 48 – 96 hours.

If the nutritive capacity of this media is to be tested for performance, it is recommended that the following ATCC organisms be evaluated for growth/inhibition.

Organism	ATCC #	Expected Growth	Special Reaction
B. fragilis	25285	24 hrs	
P. melaninogenica	25845	24 – 48 hrs	Pigment
F. nucleatum	25586	24 hrs	
C. perfringens	13124	No Growth	
P. anaerobius	27337	No Growth	
P. intermedia	25611	24 – 48 hrs	Pigment
P. gingivalis	33277	Inhibited to No Growth	

Physical Appearance: ETSA-NV should appear opaque to translucent and amber in color.

References

1. Dowell, V. R., Jr. and T. M. Hawkins. 1987. *Laboratory Methods in Anaerobic Bacteriology. CDC Laboratory Manual.* USDHHS CDC. Atlanta, GA 30333.

2. Engelkirk, P. G., Duben-Engelkirk, J. and Dowell, V. R. 1992. *Principles and Practices of Clinical Anaerobic Bacteriology*. Star Publishing Co., Belmont, CA 94002.

3. Holdeman, L. V., F. P. Cato and W. E. C. Moore. 1987. *Anaerobe Laboratory Manual*. Virginia Polytechnic Institute and State University. Blacksburg, VA 24061

4. Jousimeis-Somer, H. R., Summanen, P., Citron, D. M., Baron, E. J., Wexler, H. M. and S. M. Finegold. 2002. *Wadsworth – KYL Anaerobic Bacteriology Manual*. Star Publishing Co., Belmont, CA 94002.

5. CLSI. *Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard- Third Edition.* (2004). CLSI document M22-A3. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898.

Revision Date: 10/25/17