

HYDROLYSIS TEST MEDIA

PRODUCTS:

Tube Media:

Casein Agar	T6260
Tyrosine Agar	T7790
Xanthine Agar	T7850

PURPOSE:

These media are designed to aid in the differentiation of aerobic *Actinomycetales*, e.g., *Nocardia*, *Actinomadura*, and *Streptomyces* species by determining the presence of hydrolytic enzymes within these species.

PRINCIPLE:

Casein, Tyrosine, and Xanthine agars are useful in differentiating *Nocardia*, *Actinomadura*, and *Streptomyces* species on the basis of their patterns of hydrolysis. Certain species of aerobic actinomycetes produce hydrolytic enzymes that degrade proteins, purines, and amino acids. If hydrolysis occurs, a clearing of the media takes place.

FORMULAS: Approximate per liter formula.

- (1) **Casein Agar:**
 Skim milk.....75.0 g
 Agar.....20.0
 Final pH 7.0 ± 0.2 at 25°C

- (2) **Tyrosine Agar:**
 Beef Extract..... 3.0 g
 Pancreatic Digest of Gelatin..... 5.0
 Agar.....15.0
 Tyrosine..... 5.0
 Final pH 7.0 ± 0.2 at 25°C

- (3) **Xanthine Agar:**
 Beef Extract..... 3.0 g
 Pancreatic Digest of Gelatin..... 5.0
 Agar.....15.0
 Xanthine 4.0
 Final pH 7.0 ± 0.2 at 25°C

PRECAUTIONS:*

For in vitro diagnostic use. Observe approved biohazard precautions.

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

Limitations: It is essential that the granules of tyrosine and xanthine be kept in suspension while the media solidifies. After boiling the agar deeps, allow the molten agar to cool just short of solidification. Refrigerate the petri dishes and remove just prior to pouring. Mix the agar deeps thoroughly, and distribute evenly into the cold plate.

It is essential that a control organism be placed on each plate to determine the efficacy of the media.

The taping of plates may be necessary to reduce dehydration and possible aerial dissemination of hyphal structures.

PROCEDURE:*

Specimen Collection: Not applicable since these media are not for primary isolation. These media are used in characterizing pure cultures. Isolated organisms, established isolation techniques, and tests for purity are necessary before inoculating these media. Direct inoculation of specimens will produce erroneous results. Information on specimen collection may be found in standard reference texts.

Method of Use: See "Limitations" for plate preparation. Plates may be divided into quarters, using three isolates and a positive control for each plate. Inoculate the quadrant with a pure suspension of the test organism to the size of a quarter. Alternately, the

plates may be inoculated by transferring a piece of agar containing the organism, using a sharp sterile blade. After inoculation, seal the plate with tape and incubate at room temperature for one week or until the control is positive. Plates may be held up to three weeks before the results are interpreted.

Interpretation:

- Positive: Clearing is observed around and/or beneath the inoculum.
- Negative: No clearing is observed around and/or beneath the inoculum.

Consult standard reference texts for typical hydrolysis patterns of the actinomycetes.^{3,4}

Materials Required but Not Provided: Standard microbiological supplies and equipment are not provided.

QUALITY CONTROL:*

Microorganisms Used (ATCC #):	Expected Results:		
	Casein	Tyrosine	Xanthine
<i>Nocardia otitidiscaviarum</i> (14629)	(-)	(-)	(+)
<i>Nocardia asteroides</i> (19247)	(-)	(-)	(-)
<i>Nocardia brasiliensis</i> (19296)	(+)	(+)	(-)
<i>Streptomyces griseus</i> (10137)	(+)	(+)	(+)

Key: See "Interpretation"

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

BIBLIOGRAPHY:

1. Campbell, M. C., and J. L. Stewart, *The Medical Mycology Handbook*, John Wiley and Sons, New York, 1980.
2. Haley, L. D., and C. S. Calloway, *Laboratory Methods in Medical Mycology*, 4th ed., U.S. Department of Health, Education, and Welfare, Publication No. (CDC) 78-8361, Washington, D. C., 1978.
3. Koneman, E. W., et al., *Color Atlas and Textbook of Diagnostic Microbiology*, 1st ed., J. B. Lippincott, Philadelphia, 1979.
4. Lennette, E. H., et al., *Manual of Clinical Microbiology*, 4th ed., American Society for Microbiology, Washington D. C., 1985.

*For more detailed information, consult appropriate references.

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