TRICHOPHYTON AGARS

INTENDED USE
Remel Trichophyton Agars are solid media recommended for use in qualitative procedures for differentiation and identification of *Trichophyton* species based on nutritional requirements.

SUMMARY AND EXPLANATION
Different *Trichophyton* species resemble one another closely in culture and are difficult to differentiate on the basis of colony formation or microscopic morphology, alone. Georg and Camp developed Trichophyton Agars for differentiation and identification of *Trichophyton* isolates based on specific vitamin and amino acid requirements.[1] These media are intended for use with *Trichophyton* isolates that have been presumptively identified by gross colony characteristics and microscopic morphology.[2-5]

PRINCIPLE
Casein agar base (Trichophyton Agar #1) is the control medium used with Trichophyton Agars #2, #3, and #4 to determine if the isolate requires inositol, thiamine, or both. Ammonium nitrate agar base (Trichophyton Agar #6) is the control medium used with Trichophyton Agar #7 to determine the histidine requirement. The nicotinic acid requirement is determined using Trichophyton Agar #5 in combination with Trichophyton Agar #1 as the control medium.

REAGENTS (CLASSICAL FORMULA)*
Trichophyton Agar #1 (Casein Agar Base):

Dextrose	40.0 g
Casamino Acids	2.5 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Agar	15.0 g
Demineralized Water	1000.0 ml

Trichophyton Agar #2:

Casein Agar Base	
Inositol	50.0 mg

Trichophyton Agar #3:

Casein Agar Base	
Inositol	50.0 mg
Thiamine Hydrochloride	200.0 µg

Trichophyton Agar #4:

Casein Agar Base	
Thiamine Hydrochloride	200.0 µg

Trichophyton Agar #5:

Casein Agar Base	
Nicotinic Acid	2.0 mg

Trichophyton Agar #6 (Ammonium Nitrate Agar Base):

Dextrose	40.0 g
Monopotassium Phosphate	1.8 g
Ammonium Nitrate	1.5 g
Magnesium Sulfate	0.1 g
Agar	15.0 g
Demineralized Water	1000.0 ml

Trichophyton Agar #7:

Ammonium Nitrate Agar Base	
Histidine Hydrochloride	30.0 mg

*Adjusted as required to meet performance standards.

PROCEDURE
1. The performance of these media are dependent on a properly prepared inoculum. The test isolate should be in pure culture and grown on a nonvitamin-enriched medium such as Sabouraud Dextrose Agar or Mycobiotic Agar. Inoculate the surface of Trichophyton Agar using a small portion of the isolate to be tested. Avoid transferring agar with the inoculum since the agar may contain vitamins; the presence of extraneous nutrient materials may compromise reaction interpretation.
2. Incubate cultures at 25-30°C for 7 days for moderate- to fast-growing isolates and 10 to 14 days for slow-growing isolates. Certain species (i.e., *Trichophyton verrucosum*) demonstrate accelerated growth when incubated at 37°C. If such an isolate is suspected, incubation at 37°C may reduce the number of days required to identify the isolate.[5]
3. Record the growth in a range from +/- (trace growth) to 4+ (maximum growth). Species which require certain nutrients (i.e., thiamine, inositol, or histidine) ordinarily show approximately 2+ growth on nutrient-deprived (control) medium (i.e., Casein Agar Base and Ammonium Nitrate Agar Base).

4+ =	The tube or tubes in the series showing the **maximum growth**
1+, 2+, 3+ =	The remaining tubes in the series when compared to the tube with maximum growth (4+ growth)
+/- =	Trace of submerged growth around the inoculum

Note: To gain an appreciation for the difference between nutrient-deprived growth and maximum growth, compare the test isolate growth on each medium with that of an appropriate quality control organism on nutrient-enriched medium and control medium.[5] Moderate to heavy growth on nutrient-enriched medium in combination with little or no growth on the control medium indicates the test isolate or quality control organism requires the nutrient.

Pour Tube: Melt the pour tube in a boiling water bath and cool to 45-50°C. Mix and dispense into a sterile petri dish and proceed with the instructions above.

(Continued on back)

QUALITY CONTROL

All lot numbers of Trichophyton Agars have been tested for performance and found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. Control organisms should be selected that demonstrate a positive and negative reaction for each Trichophyton Agar tested. If aberrant quality control results are noted, patient results should not be reported.

LIMITATIONS

1. If cultures are contaminated with bacteria, the test isolate should be grown on a medium containing antibiotics for several generations to eliminate the bacteria. Many bacteria synthesize vitamins which may invalidate the test.[3]
2. When inoculating Trichophyton Agars, exercise care not to transfer agar from the primary culture medium with the test isolate. Also, the inoculum should be very small.[4,5]
3. Some *Trichophyton* species do not have a vitamin deficiency and may not be speciated using Trichophyton Agars. These species must be differentiated on the basis of a microscopic preparation and type of growth.[5]

BIBLIOGRAPHY

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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