IFU

Instructions for Use

TRICHOPHYTON AGAR

Cat. no. C51	Trichophyton Agar #1, 20x125mm Tube, 10.5ml Slant	20 tubes/box
Cat. no. C52	Trichophyton Agar #2, 20x125mm Tube, 10.5ml Slant	20 tubes/box
Cat. no. C53	Trichophyton Agar #3, 20x125mm Tube, 10.5ml Slant	20 tubes/box
Cat. no. C54	Trichophyton Agar #4, 20x125mm Tube, 10.5ml Slant	20 tubes/box
Cat. no. C55	Trichophyton Agar #5, 20x125mm Tube, 10.5ml Slant	20 tubes/box

INTENDED USE

Hardy Diagnostics Trichophyton Media numbers 1-5 are used as an aid in the identification of various *Trichophyton* species.

SUMMARY

Some species of *Trichophyton* resemble one another closely and are difficult to differentiate on the basis of colony formation or microscopic morphology. In 1957, Georg and Camp formulated the *Trichophyton* Agars based on the nutritional requirements of the various *Trichophyton* species. These nutritional tests are used to aid in the identification of *Trichophyton* species.

FORMULA

Ingredients per liter of deionized water:*

Trichophyton Agar #1:	
Casein Base Medium:	
Dextrose	40.0gm
Casamino Acids	2.5gm
Monopotassium Phosphate	1.8gm
Magnesium Sulfate	0.1gm
Agar	15.0gm

Trichophyton Agar #2:	
Casein Base Medium:	
Dextrose	40.0gm

Casamino Acids	2.5gm
Monopotassium Phosphate	1.8gm
Magnesium Sulfate	0.1gm
Inositol	50.0mg
Agar	15.0gm

Trichophyton Agar #3:	
Casein Base Medium:	
Dextrose	40.0gm
Casamino Acids	2.5gm
Monopotassium Phosphate	1.8gm
Magnesium Sulfate	0.1gm
Inositol	0.50mg
Thiamine Hydrochloride, USP	200.0mcg
Agar	15.0gm

Trichophyton Agar #4:	
Casein Base Medium:	
Dextrose	40.0gm
Casamino Acids	2.5gm
Monopotassium Phosphate	1.8gm
Magnesium Sulfate	0.1gm
Thiamine Hydrochloride, USP	200.0mcg
Agar	15.0gm

Trichophyton Agar #5:	
Casein Base Medium:	
Dextrose	40.0gm
Casamino Acids	2.5gm
Monopotassium Phosphate	1.8gm
Magnesium Sulfate	0.1gm
Nicotinic Acid	2.0mg
Agar	15.0gm

Final pH 6.8 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

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

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Not applicable since these media are not 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: Prior to inoculation, the medium should be brought to room temperature. Place a small amount (about 1mm) of the test fungus on the surface of the test media. Be careful not to transfer agar from the media on which the test isolate is growing. Place a small amount of the test fungus on Sabouraud Dextrose Agar slant. Incubate the inoculated tubes for two weeks at room temperature (15-30ºC). If results are inconclusive at that time, reincubate the cultures for two more weeks or until the Sabouraud control tube shows good growth (up to 6 weeks). The tube in the test showing maximum growth is recorded as 4+ and other tubes are read in comparison. Examine each tube grossly and quantitate by comparison of the tubes. Growth on Trichophyton Agar # 2, 3, and 4 is compared to medium 1 to determine whether the isolate requires inositol, thiamine, or both. Trichophyton Agar 5 has added nicotinic acid and is compared to medium 1.

4+ = maximum growth for the series of tests in comparison with colony growth of the other tests.
1+, 2+, 3+ = Trichophyton Agar read by comparison with the 4+ growth.
+/- = trace growth: results read by comparison to the 4+ growth.
0 = no growth.

INTERPRETATION OF RESULTS

Quantitate and compare growth with control tube. Consult appropriate references for growth patterns.

Typical Cultural Response of Certain Dermatophyte Species on Trichophyton Agars 1-5:					
Species	**1**	**2**	**3**	**4**	**5**
Trichophyton verrucosum, 84%	0	+/- to 1+	4+	0	
Trichophyton verrucosum , 16%	0	0	4+	4+	
Trichophyton schoenleinii	4+	4+	4+	4+	
Trichophyton concentricum , 50%	4+	4+	4+	4+	
Trichophyton concentricum , 50%	2+	2+	4+	4+	
Trichophyton violaceum	+/-	ND	ND	4+	
Trichophyton tonsurans	+/-	- to 1+	3+	4+	
Trichophyton rubrum	4+	4+	4+	4+	
Trichophyton mentagrophytes	4+			4+	
Trichophyton equinum	0				4+

LIMITATIONS

Pure cultures of mold must be grown on non-vitamin enriched media such as Sabouraud Dextrose Agar or Mycobiotic Agar prior to inoculation.

Cultures contaminated with bacteria must be repeatedly grown on a medium containing antimicrobials such as Mycobiotic Agar or Brain Heart Infusion (BHI) Agar with Cycloheximide and Chloramphenicol. Many bacteria synthesize vitamins which may invalidate the test.

When inoculating the Trichophyton Agars, it is important not to transfer growth substances from primary cultures to the tube media. The inoculum should be small.

Some *Trichophyton* spp. may require incubation at 35 +/- 2ºC. Consult listed references.[4]

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document

M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Trichophyton Agar #1-4					
Trichophyton mentagrophytes ATCC® 9533	G	7 days	15-30°C	Aerobic	Growth; 3 - 4+
Trichophyton equinum ATCC® 22443	G	7 days	15-30°C	Aerobic	Inhibited; 0 - +/-
Trichophyton Agar #5					
Trichophyton mentagrophytes ATCC® 9533	G	7 days	15-30°C	Aerobic	Growth; 3 - 4+
Trichophyton tonsurans ATCC® 28942	G	7 days	15-30°C	Aerobic	Inhibited; 0 - +/-

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Trichophyton Agar products should appear light amber in color.

Trichophyton mentagrophytes (ATCC® 9533) growing on Trichophyton Agar #1 (Cat. no. C51). Incubated aerobically for 7 days at 30ºC.

Trichophyton equinum (ATCC® 22443) growth inhibited on Trichophyton Agar #1 (Cat. no. C51). Incubated aerobically for 7 days at 30ºC.

REFERENCES

1. Campbell, M.C. and J.L. Stewart. 1980. *The Medical Mycology Handbook*, John Wiley & Sons, New York, NY.

2. Georg, L.K. and L.B. Camp. 1957. *J. Bacteriol*.; 74:113.

3. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

4. Haley, L.D. and C.S. Calloway. 1984. *Laboratory Methods in Medical Mycology*, 4th ed. U.S. Department of Health and Welfare, *Manual of Clinical Mycology*, 5th ed. ASM, Washington, D.C.

5. Difco Manual, 10th ed. 1984. Difco Laboratories, Detroit, Michigan.

6. Hugh, R., and Leifson, J. 1957. *J. Bacteriol*.; 74:113.

ATCC is a registered trademark of the American Type Culture Collection.

IFU-10803[C]

1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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