IFU

Instructions for Use

CORN MEAL AGAR WITH TWEEN® 80

Cat. no. W10	Corn Meal Agar with Tween® 80, 15x100mm Plate, 26ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Corn Meal Agar with Tween® 80 is recommended for use in the cultivation of fungi and for the inducement of chlamydospore formation by *Candida albicans*.

SUMMARY

Corn Meal Agar is an enrichment medium developed by Hazen and Reed for use in the cultivation of fungi.[11] Walker and Huppert, in 1960, found that the addition of Tween® 80 to Corn Meal Agar resulted in rapid and abundant chlamydospore formation.[10] The medium consists of corn meal infusion and agar. Growth nutrients are provided by the infusion product. Tween® 80, a mixture of oleic esters, stimulates the production of chlamydospores by *C. albicans*, *C. stellatoidea* and occasionally *C. tropicalis*.[2,10]

Corn Meal Agar with Tween® 80 tubed media are used for the maintenance of fungal stock cultures.

FORMULA

Ingredients per liter of deionized water:*

Corn Meal Infusion	2.0gm
Tween ® 80	7.0ml
Agar	15.0gm

Final pH 6.2 +/- 0.3 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. Store products away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Products are 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: This media is not intended for primary isolation. It is for use in characterizing pure cultures. Consult listed references for information regarding the processing and inoculation of specimens.[1,2,4-6]

1. Using an inoculating needle, obtain visible paste of the organism. Draw the needle through the agar making two perpendicular lines in the shape of an "x".

2. Flame a coverslip and allow it to cool. Once coverslip has cooled, place over the central area of the "x" in order to reduce oxygen tension. Reduced oxygen tension stimulates chlamydospore production. Leave some growth uncovered.

3. Seal the plate with tape or MycoSeals™ (Cat. no. SS9225) and incubate aerobically at room temperature (25-30ºC) for up to 72 hours in the dark. Examine daily for typical colonial growth and morphology.

4. Invert the plate and examine microscopically using a low power objective (10X). View along the edge of the coverslip for detection of chlamydospore formation.

5. Follow steps one through four of the above procedure for the examination of sporulation of molds.[6,7] Incubate until mold is visible and mount coverslip in glycine. Examine microscopically for characteristic structures.

INTERPRETATION OF RESULTS

Using low power magnification, examine for the presence of budding cells, hyphae, blastospores, and chlamydospores. Most strains of *C. albicans* and *C. stellatoidea* form typical chlamydospores after 24-48 hours incubation. Examine daily for up to four days.[8] *C. dubliniensis* will also form chlamydospores, but in clusters, rather than singles as with the *C. albicans*.

LIMITATIONS

Chlamydospore formation is inhibited at 30-37ºC.[12] A temperature of 25ºC is recommended for the best results.

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

A non-selective and selective medium should be inoculated for isolation of fungi from potentially contaminated specimens.

Repeated subculturing of some *Candida* strains result in a loss of their ability to produce chlamydospores.

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, swabs, applicator sticks, other culture media, incinerators, 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	
Candida albicans ATCC® 10231	C	24-72hr	15-30°C	Aerobic	Growth; hyphae, budding cells, and chlamydospores seen
Candida glabrata ATCC® 66032	C	24-72hr	15-30°C	Aerobic	Growth; no chlamydospores seen

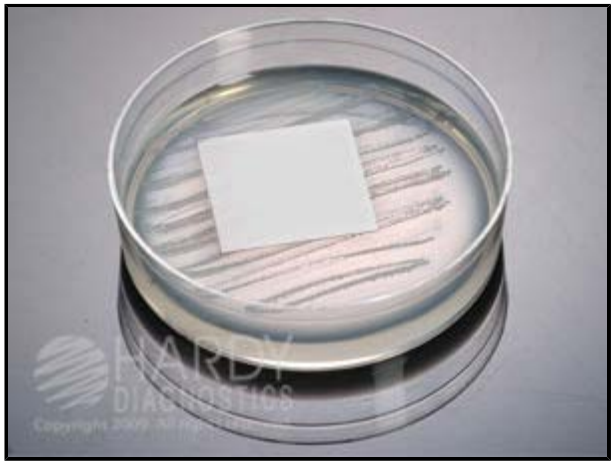
* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

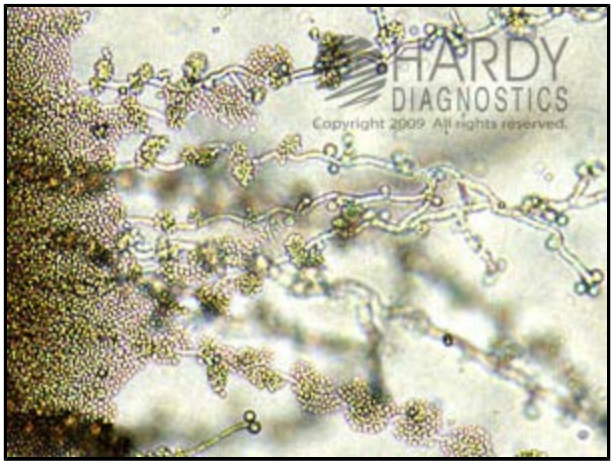
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory 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

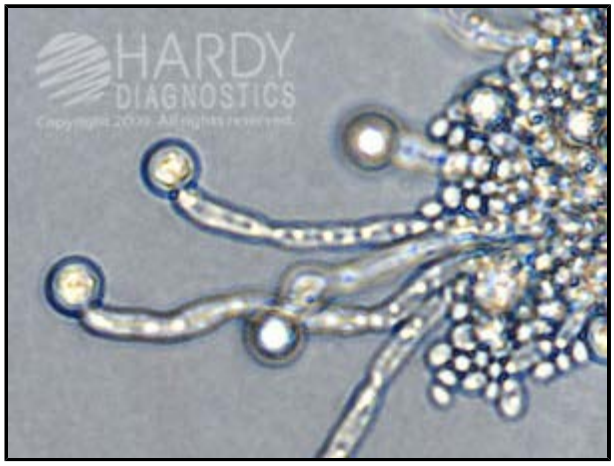
Corn Meal Agar with Tween® 80 should appear slightly opalescent, and white in color.



Candida albicans (ATCC® 10231) colonies growing on Corn Meal Agar with Tween® 80. Incubated aerobically for 72 hours at 15-30ºC. Shown with coverslip.



Microscopic image at 100x of chlamydospores from *Candida albicans* (ATCC® 10231) grown on Corn Meal Agar with Tween® 80.



Microscopic image at 500x of chlamydospores from *Candida albicans* (ATCC® 10231) grown on Corn Meal Agar with Tween®80.

Uninoculated plate of Corn Meal Agar with Tween® 80.

REFERENCES

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10. Walker and Huppert. 1960. *Tech. Bull. Reg. Med. Technol.*; 30:10.

11. Hazen, E.L. and F.C. Reed. 1955. *Monograph Laboratory Identification of Pathogenic Fungi Simplified*, American Lecture Series, Publ. No. 253, p. 100. Charles C. Thomas, Springfield, IL.

12. Larone, D.H. *Medically Important Fungi: A Guide to Identification*, American Society for Microbiology. Washington, D.C.

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

HDQA 2207F [D]