IFU

Instructions for Use

GERM TUBE CRYO™

Cat. no. Z217	Germ Tube Cryo™ , 2ml Cryogenic Vial, 0.5ml Fill	20 vials/container

INTENDED USE

Hardy Diagnostics Germ Tube Cryo™ is a ready-to-use, single use vial to assist in the preliminary identification of *Candida albicans* by germ tube formation.

SUMMARY

The most frequently found yeast pathogen in clinical diagnosis is *Candida albicans* , and it has been implicated in a variety of clinical disorders. The rapid preliminary clinical identification of this yeast is usually based on the formation of hyphae by the yeast cell that are half its width, up to three or four times the length of the cell, and with no constriction seen between the cell and the hyphal growth.

Known as a germ tube, the growth occurs when the yeast is incubated in a serum medium at 35ºC. for one to two hours. Human, sheep, rabbit, and fetal (or newborn) calf serum have been used as media for this test with varying results, as well as various peptone media, saliva, egg yolks, and tissue culture media. Human serum methods are no longer recommended due to the potential of disease transmission of bloodborne pathogens. Standardization of non-human serum substitutes have been reached at this time. The unique formula of Hardy Diagnostics Germ Tube Cryo™ increases the stability of the product so that it can be shipped without refrigeration during transit, even though it is stored in the frozen state.

REAGENT FORMULA

The Germ Tube Cryo™ contains a ready-to-use solution of Bovine Serum and Tryptic Soy Broth in a single test quantity. Additional supplements have been added which promote or induce rapid germ tube formation.

STORAGE AND SHELF LIFE

Storage: **Upon receipt store vials at less than -10ºC.** The product may be shipped without refrigeration if transit time is less than one week. Product should not be used if there are any signs of deterioration, contamination, or if the expiration date has passed.

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 product is not intended for primary isolation of patient specimens. The Germ Tube Cryo™ may be used as a rapid presumptive identification method, in conjunction with other biochemical and/or serological tests to identify cultures of isolated yeasts. For optimum results, use colonies taken from SabDex Agar (Cat. no. W70) or Blood Agar (Cat. no. A10) that have been incubated for 24 to 48 hours at 30ºC.

Method of Use: Thaw the Germ Tube Cryo™ to room temperature (15-30ºC.) and label the vial appropriately. With a sterile loop, applicator stick, or pasteur pipette, lightly touch a colony. The inoculum must be very light (resulting in approximately 10^5 cells per ml). Emulsify cells in the medium. **Do not** heavily inoculate the vial. Increased amounts of inoculum decrease germ tube production and can cause false-negatives.

Cap may be left off of vial and pipette left in tube while incubating. Incubate aerobically in an incubator or heat block for no longer than two hours at 35ºC. Microscopic examination may begin at 30 minutes. Examine culture for presence of germ tubes by placing one drop of media on a slide, covering it with a coverslip, and examining on high dry magnification (400X). There should not be more than six cells per view, otherwise the inoculum was too heavy.

INTERPRETATION OF RESULTS

A positive test for *Candida albicans* is determined by the presence of a thin, tube-like structure half the width and approximately three to four times the length of the yeast cell. No constriction should be seen at the germ tube/cell wall interface. *C. tropicalis* may produce germ tube-like structures but there is a definite constriction where the structure joins the blastoconidium. *C. dubliniensis* is also germ tube positive.

LIMITATIONS

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.

If medium is incubated longer than two hours, other types of *Candida* yeasts may start producing germ tubes, thus resulting in possible false-positives.

Medium must be sterile and isolate must be pure, as germ tube production may be inhibited or delayed in contaminated serum media.

A heavy inoculum (greater than six cells per 400X field) can cause false-negatives.

It has been shown that 5% of routine isolates of *C. albicans* test negative for germ tube production, although it is extremely rare for *C. albicans* to be negative for both germ tube and chlamydospore formation. Other *Candida* spp.

such as rare isolates of *Candida tropicalis* may produce similar germ tube structures with constrictions.

The Germ Tube Cryo™ is a rapid presumptive method to detect the presence of *Candida albicans* . It is only a part of an overall process of identification, which includes biochemical and serological tests that will completely identify the organism. These procedures may be found in the appropriate texts.[1,2,4]

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	**Reaction**
Candida albicans ATCC® 10231	Positive; germ tubes formed within 2 hours
Candida tropicalis ATCC® 750	Negative; no germ tubes formed within 2 hours

* 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

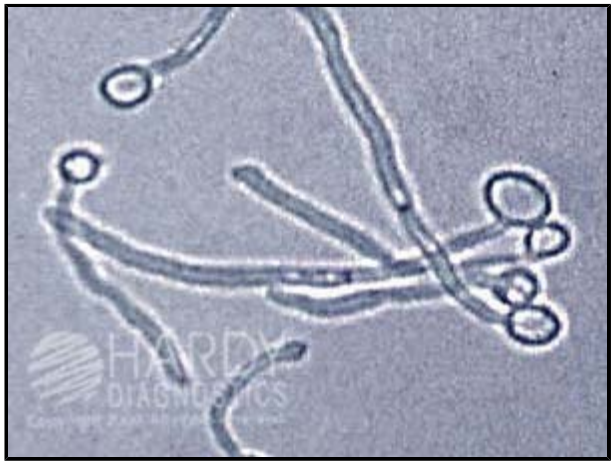
Germ Tube Cryo™ should appear clear, and medium amber in color.

REFERENCES

1. Versalovic, J., et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

2. Tille, P.M., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

3. Howard, B.J., et al. 1994. *Clinical and Pathogenic Microbiology* , 2nd ed. C.V. Mosby Company, St. Louis, MI.



Microscopic image at 500x of germ tubes formed by *Candida albicans* (ATCC® 10231).

4. Isenberg, H.D., ed. *Clinical Microbiology Procedures Handbook* , Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology* . J.B. Lippincott Company, Philadelphia, PA.

6. Kwon-Chung, K.J. and J.E. Bennett. 1992. *Medical Mycology* . Lea and Febiger, Malvern, PA.

7. Berardinelli, S. and D.J. Opheim. 1985. New germ tube induction medium for the identification of *Candida albicans* . J. Clin. Microbiol.; 122:861-862.

8. *Commission on Laboratory Accreditation* , *Laboratory Accreditation Program Microbiology Checklist* . College of American Pathologists. Rev. 9/30/2004.

9. Centers for Medicare and Medicaid, *Appendix C, Survey Procedures and Interpretive Guidelines for Laboratories and Laboratory Services* . Subpart K - Quality System for Non-Waived Testing. 493;1200-1265. www.cms.hhs.gov/clia .

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

HDQA 2207F [D]