IFU

Instructions for Use

HardyCHROM™ Candida + auris

Cat. no. G343	HardyCHROM™ Candida + auris, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics HardyCHROM™ Candida + auris is recommended as a medium for the primary, selective isolation and differential identification of *Candida* species. The medium allows for the differentiation of *C. albicans*, *C. tropicalis*, *C. krusei*, and *C. auris* based on colony morphology, color, and positive UV fluorescence.

SUMMARY

HardyCHROM™ Candida + auris is a selective and differential medium containing chromogenic substrates. After degradation by specific enzymes, the substrates release different colored compounds. Certain species or groups of organisms can then be differentiated with a minimum number of confirmatory tests.

Colonies of *C. albicans* will appear green to dark metallic green. Colonies of *C. tropicalis* will appear medium blue to dark metallic blue with a blue halo. Colonies of *C. krusei* will appear flat, often rough or crenated, and pink to medium pink in color. Colonies of *C. auris* will appear white with a characteristic teal to teal-green "bullseye" center. Other species will appear pink, often with a darker mauve center (e.g. *C. glabrata* and other species). Other yeasts may appear white to pink. Most typical yeast colonies tested for fluorescence at 48 hours using a UV lamp at 365nm will be negative, except for *C. auris* which will be positive at 48-72 hours.

Additionally, HardyCHROM™ Candida + auris can be used in conjunction with Rapid Trehalose Broth (Cat. no. Z205) or GlabrataQuick™ (Cat. no. Z298) to aid in the identification of *C. glabrata* . When HardyCHROM™ Candida + auris is used as the primary plating medium, only colonies that morphologically (pink, often with a darker mauve center) resemble *C. glabrata* should be tested for trehalose assimilation.

Candida auris is an emerging healthcare-associated pathogen that represents a serious multi-drug resistant (MDR) global threat. Though the organism often presents fewer virulence factors than *Candida albicans*, *C. auris* may persistently colonize the skin and hospital environment, making its transmission within and between healthcare settings more difficult to control.[(11)] *C. auris* fungemia results in a wide range of mortality rates (from 32%-66%), depending upon the patient's overall condition, underlying disease, geographic region, access to medical care, and age.[(11)] A review of the organism's genome demonstrates that it harbors genes well characterized as virulence factors in other *Candida* species, as well as genes for biofilm production and MDR transcription factors.[(11)]

HardyCHROM™ Candida + auris contains glucose and selected peptones as a nutrient supply. Chromogenic substrates are incorporated to enable the production of different colored compounds when degraded by specific enzymes formed by the yeast. Chloramphenicol is added as an inhibitory agent against the growth of most bacteria, which may be present in the sample. Agar is the solidifying agent.

FORMULA

Ingredients per liter of deionized water:*

Glucose	20.0gm
Peptone	10.0gm
Chromogenic Mixture	2.15gm
Titanium Dioxide	2.0gm
Chloramphenicol	0.5gm
Agar	15.0gm

Final pH 6.1 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at 2-8ºC 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. 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: Consult listed references for information on specimen collection.[1-8] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If a delay in processing is anticipated, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation.

Consult the listed references for information regarding the processing of specimens.[1-9]

Protect media from light during storage and incubation as the product is light sensitive.

Method of Use:

1. Allow plates to warm to room temperature. The agar surface should be dry prior to inoculating.
2. Inoculate and streak the specimen as soon as possible after collection to obtain well isolated colonies. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface. Streak for isolation with a sterile loop.
3. Incubate plates in an inverted position, protected from the light, aerobically at 35ºC. with increased humidity.
4. Examine plates after 24, 48, and 72 hours of incubation. *

*In instances where *C. auris* is suspected, it is recommended to extend the incubation period to at least 72 hours. See the Limitations section for more information.

INTERPRETATION OF RESULTS

Examine plates for colonies showing typical morphology and color. Use a 365nm wavelength handheld UV Lamp (Cat. no. UVL56 or LSS3) to detect colony fluorescence. These handheld lamps require that the room lights be turned off, since ambient light will interfere with fluorescence detection. Alternatively, a dark viewing box (Cat. no. CM10A) with its companion UV lamp (Cat. no. EA160) may be used so that the room lights will not need to be turned off.

CAUTION: Not all UV wavelengths are capable of producing sufficient fluorescence effects. It is important to use a UV light with a wavelength at or near 365nm, one with higher power (in watts, not lumens), and one that is high efficiency. Use of UV lights not meeting these criteria will fail to produce sufficient fluorescence. Most inexpensive battery operated LED UV lights produce light at multiple wavelengths, use less watts, and/or low power, and are thus **not acceptable** and will produce erroneous results. Cat. no. LSS3 is an exception and has been verified to work well. Please do not use cheaper versions.

Tips for using fluorescence

1. Use a 365nm handheld UV lamp (Cat. no. UVL56) or (Cat. no. LSS3) to detect colony fluorescence. See CAUTION above regarding inexpensive handheld UV lights. Alternatively, a dark viewing box with its compatible UV lamp may be used as described above. Viewing must be done in the dark.
2. Hold the lamp directly over isolated colonies on the plate, approximately 3 to 4 inches (7 to 10cm) away.
3. Isolated colonies of *Candida auris* will fluoresce a greenish white glow.
4. Only well isolated colonies will fluoresce. Colonies in areas of confluent growth will not.
5. Fluorescence will fade over time, as colonies develop to a darker teal/green pigment.

24 Hours – Incubate at 35°C., then observe plates for a teal bullseye pattern in isolated colonies. Isolated colonies with no color development may require an additional 48 hours of incubation.

48 Hours – Observe plates for a teal bullseye pattern in isolated colonies. If teal bullseye pattern is present, examine under 365nm UV light in the dark for fluorescence (see instructions above). Isolated colonies with no color development may require an additional 24 hours of incubation.

72 Hours – Observe plates for teal bullseye pattern in isolated colonies. If teal bullseye pattern is present, examine under 365nm UV light in the dark for fluorescence. As the color of the colonies intensifies, the fluorescence will fade. Discard plates after 72 hours.

Some strains may show sufficient growth and color development when read at 24 hours; however, all plates should be incubated for at least 72 hours to allow for adequate color development and to detect fluorescence. Colors will intensify with age, but fluorescence will fade over time. Therefore, check for fluorescence at 48 hours.

Candida albicans - A medium size, smooth, green to dark metallic green colored colony at 48 hours is identified as *C. albicans*. Colonies are negative for UV fluorescence at 48 hours.

Candida tropicalis - A medium size, smooth, medium blue to dark metallic blue colored colony, with a blue halo, at 48

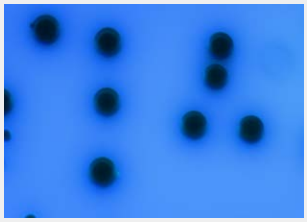
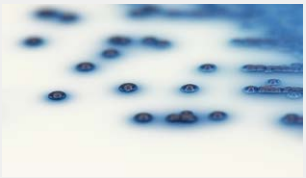
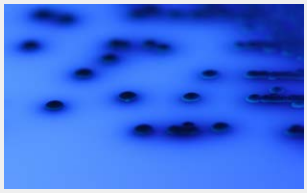
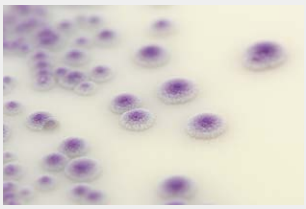
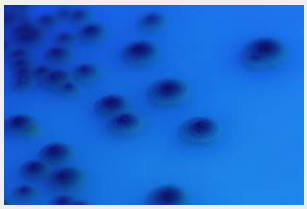
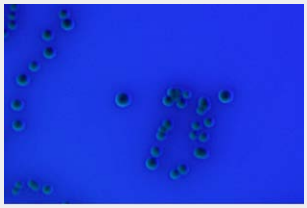
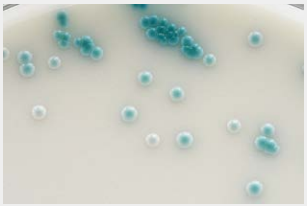
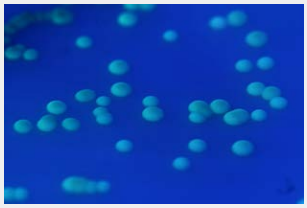
hours is identified as *C. tropicalis*. Colonies are negative for UV fluorescence at 48 hours.

Candida krusei - A large, flat, spreading, often rough or crenated, pink to medium pink colored colony is identified as *C. krusei*. Colonies are negative for UV fluorescence at 48 hours.

Candida glabrata - A medium size, smooth, pink colored colony, often with a darker mauve center, is presumptively identified as *C. glabrata*. A Rapid Trehalose test is needed (see "Limitations" below). Colonies are negative for UV fluorescence at 48 hours.

Candida auris - A medium size, smooth, white with teal to teal-green “bullseye” center at 48-72 hours is identified as *C. auris*. Fluorescence may be absent in areas of heavy confluent growth of *C. auris*. However, well isolated colonies will fluoresce. Colonies are positive for UV fluorescence at 48-72 hours. Only *C. auris* will develop both teal bullseye colonies and fluorescence. See the Limitations section for more information.

Other yeast are generally small, white to pink colored colonies. Colonies are negative for UV fluorescence at 48 hours.

Organism	48hr Reaction	48hr Appearance	48hr Fluorescence
Candida albicans	Medium, smooth, green to dark metallic green colonies; negative Fluorescence		
Candida tropicalis	Medium, smooth, medium blue to dark metallic blue colonies, with a blue halo; negative Fluorescence		
Candida krusei	Large, flat, spreading, often rough or crenated, pink to medium pink colored colonies; negative Fluorescence		
*Candida glabrata**	Medium, smooth, pink colonies, often with a darker mauve center; negative Fluorescence		
*Candida auris***	Medium, smooth white colonies with a teal to teal-green "bullseye" center; positive Fluorescence		

* A Rapid Trehalose test is needed (see "Limitations" below) to confirm *C. glabrata*.

** Full color development may take up to 72 hours for slow growing strains or for strains from mixed specimens.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry (MALDI-TOF) testing be performed on colonies from pure culture for complete identification.

Some strains of *C. auris* may show a delayed color reaction and UV fluorescence in mixed specimens. This may also occur when the initial inoculum is low. In instances where delayed growth of *C. auris* is suspected, it is recommended to extend the incubation period to at least 72 hours.

Some strains of *C. auris* recovered in the presence of other yeasts, such as *C. glabrata* or *C. krusei*, may exhibit a uniform teal to teal-green coloration instead of the characteristic "bullseye" colony center. In these instances, and as the *C. auris* colony color darkens over time, fluorescence is no longer detected. Therefore, it is recommended to read the cultures for fluorescence at 48 hours and, if negative, again at 72 hours, since fluorescence can fade over time.

Other rare *Candida* species, as well as non-clinical yeast species, may exhibit fluorescence under UV light, but will not exhibit the characteristic white colonies with teal to teal-green “bullseye” centers at 48 to 72 hours indicative of *C. auris*.

C. haemulonii, which has been commonly misidentified for *C. auris*, exhibits fluorescence under UV light but produces white colonies at 48 hours. *C. haemulonii* will eventually produce light pink or mauve colonies when incubated longer than 48 hours. Colonies will be positive for UV fluorescence.

Colonies of *C. glabrata* may show positive UV fluorescence at 24 hours, but the reaction will disappear by 48 hours.

Candida spp., other than *C. glabrata*, may present white to pink colored colonies on HardyCHROM™ Candida + auris which is why *C. glabrata* must be confirmed using Trehalose assimilation. Refer to the Rapid Trehalose Broth (Cat. no. Z205) or GlabrataQuick™ (Cat. no. Z298) technical information sheet for additional information concerning the definitive identification of *C. glabrata*. Colonies that are 24 hours old may be used to test for rapid Trehalose assimilation.

Isolates of *C. dubliniensis* will grow on this medium and will produce colors similar to or slightly different from *C. albicans* on primary isolation. The color variation will be lost upon subculture, so additional testing may be required to differentiate the two species. Both *C. dubliniensis* and *C. albicans* are germ tube positive, but *C. dubliniensis* can generally be differentiated from *C. albicans* by susceptibility to fluconazole, demonstration of TTC reduction (i.e. production of red colonies on media containing 2,3,5-triphenyl-tetrazolium), and poor to no growth at 45°C.[13]

C. parapsilosis and *C. duobushaemulonii* may develop teal coloration in areas with heavy growth (1st and 2nd quadrants), as well as fluorescence under UV light at 48 hours. Some strains of *C. parapsilosis* and *C. duobushaemulonii* will exhibit the characteristic teal “bullseye” colonies, sometimes mixed with mauve “bullseye” colonies with fluorescence under UV light at 72 hours.

This product is not intended for the isolation and identification of *Cryptococcus* spp.

Fluorescence must be read in a darkened environment with a 365nm wavelength UV lamp of adequate power (see “Tips for Using Fluorescence” above).

Color-blind individuals may encounter difficulty in distinguishing the color differences on HardyCHROM™ Candida + auris.

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, UV lamps, applicator sticks, incinerators, handheld UV lamp (Cat. no. UVL56 or LSS3) or dark viewing box (Cat. no. CM10A) with compatible UV lamp (Cat. no. EA160), Rapid Trehalose Broth (Cat. no. Z205), GlabrataQuick™ (Cat. no. Z298), 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	B	24-48hr	35°C	Aerobic	Growth; smooth, emerald green to dark metallic green colonies; negative UV fluorescence
Candida tropicalis ATCC® 750	B	24-48hr	35°C	Aerobic	Growth; smooth, medium blue to dark metallic blue colonies with a blue halo; negative UV fluorescence
Candida krusei ATCC® 14243	B	24-48hr	35°C	Aerobic	Growth; flat, pink to medium pink, large spreading, rough, crenated colonies; negative UV fluorescence
Candida glabrata ATCC® 66032	B	24-48hr	35°C	Aerobic	Growth; smooth pink colonies, often with a darker mauve center; negative UV fluorescence***
Candida auris CDC B11903	B	24-48hr	35°C	Aerobic	Growth; smooth, white colonies with teal to teal-green bullseye" center; positive UV fluorescence
Escherichia coli ATCC® 25922	B	24hr	35°C	Aerobic	Partial to complete inhibition; negative UV fluorescence

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

** QC incubation period based on inoculum of pure isolates.

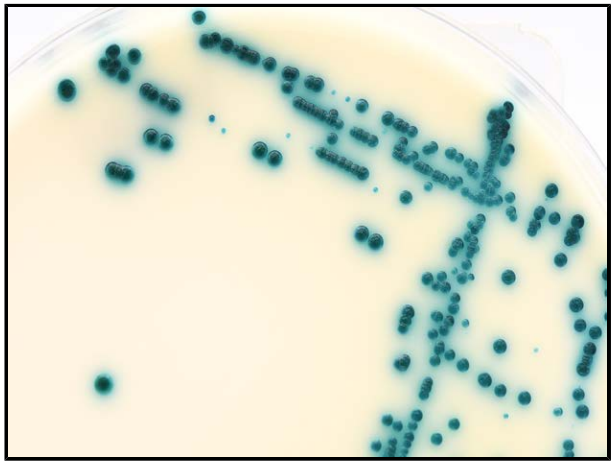
*** Fluorescence detected at 24hrs and disappears by 48hrs.

USER QUALITY CONTROL

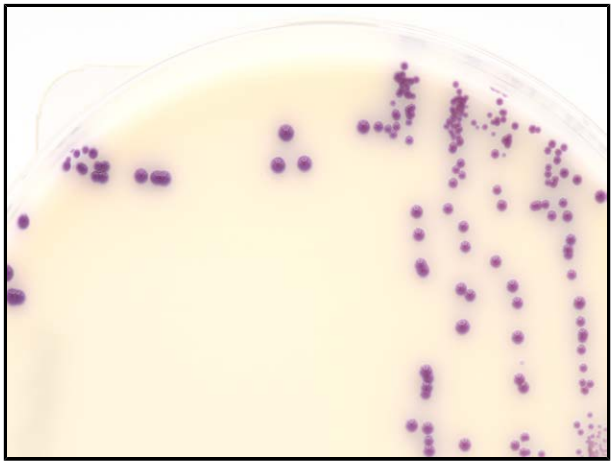
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory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

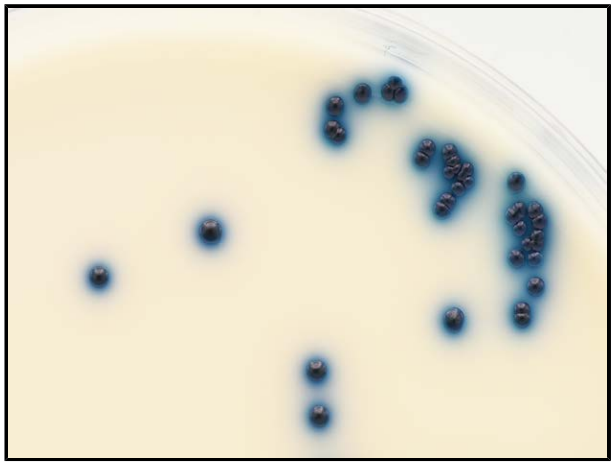
HardyCHROM™ Candida + auris should appear opaque and white in color.



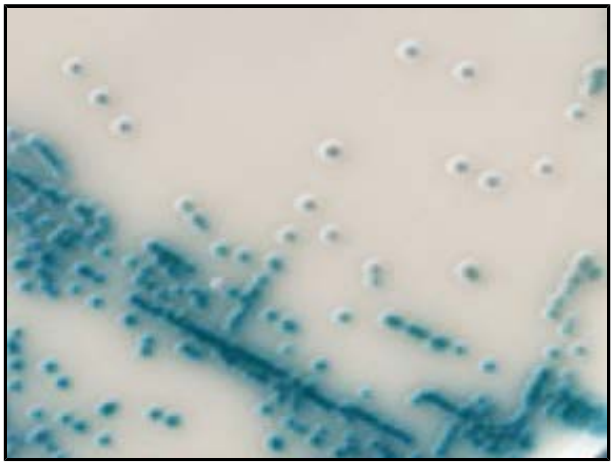
Candida albicans (ATCC® 10231) colonies growing on HardyCHROM™ Candida + auris (Cat. no. G343). Incubated aerobically for 48 hours at 35ºC.



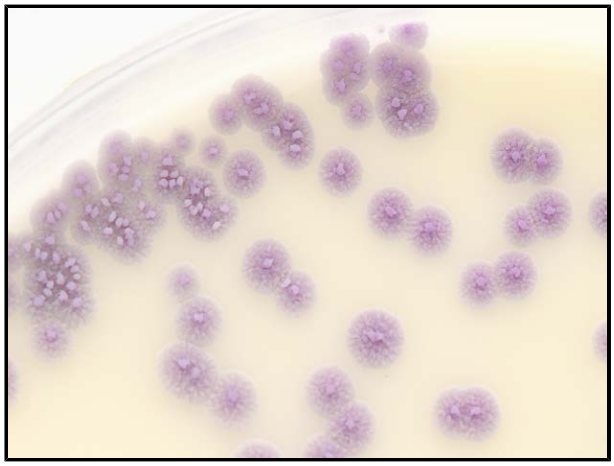
Candida glabrata (ATCC® 66032) colonies growing on HardyCHROM™ Candida + auris (Cat. no. G343). Incubated aerobically for 48 hours at 35ºC.



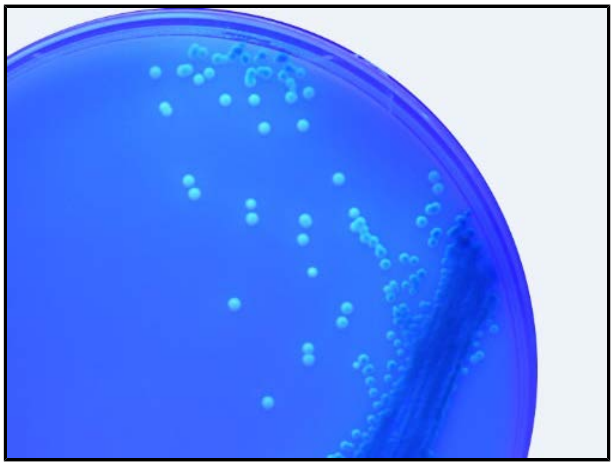
Candida tropicalis (ATCC® 750) colonies growing on HardyCHROM™ Candida + auris (Cat. no. G343). Incubated aerobically for 48 hours at 35ºC.



Candida auris (CDC B11903) colonies growing on HardyCHROM™ Candida + auris (Cat. no. G343). Incubated aerobically for 48 hours at 35ºC.



Candida krusei (ATCC® 14243) colonies growing on HardyCHROM™ Candida + auris (Cat. no. G343). Incubated aerobically for 48 hours at 35ºC.



Candida auris (CDC B11903) colonies growing on HardyCHROM™ Candida + auris (Cat. no. G343) showing positive UV fluorescence. Incubated aerobically for 48 hours at 35ºC.

REFERENCES

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5. Haley, L.D., et al. 1980. *Cumitech 11; Practical Methods for Culture and Identification of Fungi in the Clinical Microbiology Laboratory*, Coordinating ed., J.C. Sherris. American Society for Microbiology, Washington, D.C.

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12. Centers for Medicare & Medicaid Services (CMS). Individualized Quality Control Plan (IQCP).

13. Giammanco GM, G. Pizzo, S. Pecorella, S. Distefano, V. Pecoraro, M.E. Milici. April 2002. Identification of *Candida dubliniensis* among oral yeast isolates from an Italian population of human immunodeficiency virus-infected (HIV+) subjects. *Oral Microbiol Immunol*. 17(2):89-94.

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

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