



Instructions for Use

HardyCHROM™ UTI

Cat. no. G313	HardyCHROM™ UTI, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. G313BX	HardyCHROM™ UTI, 15x100mm Plate, 18ml	100 plates/box
Cat. no. J119	Blood Agar / HardyCHROM™ UTI, 15x100mm Biplate, 10ml/10ml	10 plates/bag

INTENDED USE

HardyCHROM™ UTI is a non-selective chromogenic medium recommended for the cultivation, differentiation and enumeration of urinary tract pathogens based on colony color and morphology.

SUMMARY

There are a number of organisms routinely isolated from urinary tract infections (UTI). Most UTIs are caused by *Escherichia coli* alone, or in combination with other organisms. The most frequently isolated species produce characteristic enzymes. Chromogenic substrates (chromogens) incorporated into HardyCHROM™ UTI produce different colored compounds when they are degraded by specific microbial enzymes. Thus, HardyCHROM™ UTI can be used for the cultivation and differentiation of various groups of organisms with only a minimum number of confirmatory tests.

Peptones supply the necessary nutrients, and the mixture of chromogens permits detection and differentiation of the isolated organisms. This medium contains no inhibitory substances and is not selective. The swarming of *Proteus* is partially to completely inhibited.

FORMULA

Ingredients per liter of deionized water:*

Peptones	16.0gm
Chromogenic Mixture	5.0gm
Agar	15.0gm

Final pH 6.9 +/- 0.3 at 25 degrees C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8 degrees C. 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.

Chromogens are especially light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date applies to the product in its intact packaging when stored as directed.

This product has the following shelf life from the date of manufacture:

90 Days:	G313	HardyCHROM™ UTI
	G313BX	HardyCHROM™ UTI
	J119	Blood Agar / HardyCHROM™ UTI

Refer to the keyword "Storage", in the Hardy Diagnostics' software program HUGO™, for more information on storing culture media.

PRECAUTIONS

This product is for *in vitro* diagnostic use only and 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". The "Guideline for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline*.

Sterilize all biohazard waste before disposal.

Refer to the keyword "Precautions", in the Hardy Diagnostics' software program HUGO™, for more information regarding general precautions when using culture media.

Refer to the keyword "MSDS", in the Hardy Diagnostics' software program HUGO™, for more information on handling potentially hazardous material.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.⁽²⁻⁴⁾ Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be refrigerated until inoculation.

Consult the listed references for information regarding the processing of specimens.⁽¹⁻⁵⁾

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

Method of Use: Allow the plates to warm to room temperature. The agar surface should be to dry prior to inoculating. Inoculate and streak the specimen as soon as possible after collection. For quantitative testing streak plate with 0.01 ml (Cat. no. HS10R) or 0.001ml calibrated loop (Cat. no. HS1R). Incubate plates in an inverted position, aerobically at 35 +/- 2 degrees C. for no less than 24 hours. Examine plates for colonies showing typical morphology and color after 24 hours, but no later than 36 hours.

Do not incubate in an atmosphere supplemented with CO₂.

INTERPRETATION OF RESULTS

After incubation, the plates should show isolated colonies. Isolated colonies are necessary for demonstration of typical color and morphology.

Escherichia coli produces medium to large sized colonies that are rose to magenta in color, with darker pink centers. No further testing is needed.

Colonies that resemble *E. coli* (pink to rose), but are small or pinpoint in size, require further identification procedures such as the Indole Spot Test (DMACA, Cat. no. Z65). See "Limitations" section below.

Enterococcus spp. appear as small, teal to turquoise colored colonies. No further testing is needed.

Proteus, *Morganella*, and *Providencia* spp. produce clear to light yellow colonies with golden-orange halo diffused through surrounding media. Additionally, approximately 50% of *Proteus vulgaris* isolates will produce blue-green or green colonies with a golden-orange halo. *Proteus vulgaris* can be identified by a positive spot indole test (Cat. no. Z65). Further biochemical tests are needed for complete identification of the other members of this group. Indole Spot Test (Cat. no. Z65) may be performed from the plate. H₂S production and ornithine decarboxylase (Cat. no. Y44) permit differentiation of the genera.

Klebsiella, *Enterobacter*, and *Serratia* spp. produce large, deep blue or dark indigo colonies. *Citrobacter* spp. produce dark blue colonies often with a rose halo in the surrounding media. Further biochemical tests such as the Microgen GN-A Panel (Cat. no. MID64) are needed for complete identification.

Pseudomonas spp. produce colorless to light yellow-green, translucent colonies which may have a slight iridescence with crinkled edges. Further biochemical tests, including an oxidase test (Cat. no. Z93) may be needed for complete identification.

Staphylococcus aureus produce opaque, cream to white colored colonies. **Note:** Colonies may turn pink after 72 hours. Further tests (StaphTEX™, Cat. no. ST50) are needed for complete identification.

Staphylococcus saprophyticus produce opaque, pink colonies. Further tests, such as novobiocin-resistance (Cat. no. Z7291), are needed for complete identification.

Staphylococcus epidermidis grows as small, white colonies. Further biochemical tests are needed for complete identification.

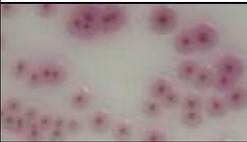
Candida albicans, *Candida tropicalis*, and *Candida glabrata* produce small, opaque, white, moist colonies. Further biochemical tests such as AlbiQuick™ (Cat. no. Z121) or HardyCHROM™ Candida (Cat. no. G301) are needed for complete identification.

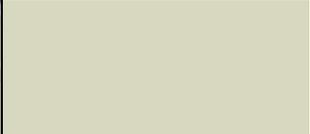
Candida krusei appears as small, white, dry colonies which have a rough appearance. Further biochemical tests are needed for complete identification.

Listeria monocytogenes or other *Listeria* spp. may be present in urine. Colonies of *Listeria* are very small, blue to blue-green colonies. Perform a Gram stain of organisms producing small, blue to blue-green colonies that are PYR-negative. The presence of gram-positive bacilli is suggestive of *Listeria* spp. but further biochemical tests are necessary for complete identification.

Streptococcus agalactiae isolated from urine appears as very small clear blue colonies, very small clear white colonies or very small pink or pink-blue colonies. Further tests, such as Strep B Carrot Broth™ (Cat. no. Z140), are needed for complete identification.

For organisms other than *E. coli* and *Enterococcus* spp. biochemical tests should be performed on colonies from pure cultures for complete identification. Use a filter paper to perform rapid tests. Do not apply any detection reagents directly on the colonies growing on the medium.

Organism	Description	Photo	Color
<i>E. coli</i>	rose to magenta colonies with darker pink centers		
<i>Klebsiella</i> , <i>Enterobacter</i> , and <i>Serratia</i> spp.	deep blue or dark indigo colonies		
<i>Enterococcus</i> spp.	teal to turquoise colonies		
<i>Proteus</i> , <i>Morganella</i> , and <i>Providencia</i> spp.	clear to light yellow colonies with golden-orange halo in the surrounding media (some <i>Proteus vulgaris</i> colonies will be blue-green)		
<i>Staphylococcus aureus</i>	opaque, cream to white colored colonies		
<i>Pseudomonas</i> spp.	colorless to light yellow-green colonies		

<i>Staphylococcus saprophyticus</i>	opaque, pink colonies		
<i>Candida albicans</i> , <i>Candida krusei</i> , <i>Candida tropicalis</i> , and <i>Candida glabrata</i>	small, opaque, white, moist colonies (<i>C. krusei</i> will be a rough colony)		
<i>Citrobacter</i> spp.	dark blue colonies often with a rose halo in the surrounding media		

LIMITATIONS

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

Use of this medium for non-clinical or clinical specimens other than urine has not been validated.

Some rare strains of *C. freundii* may produce small, pink or rose colored colonies, with color similar to *E. coli*. To prevent misidentification, a rapid Indole Spot Test (Cat. no. Z65) may be performed since *C. freundii* is indole-negative and *E. coli* is indole-positive.

Aerococcus urinae does not grow well on this medium. After 48 hours the colonies are very small to pinpoint and are colorless.

Corynebacterium renale does not grow on this medium (48 hours).

Do not use Kovacs Indole Reagent on dark rose or pink colonies as the colony color may interfere with the red color of a positive indole reaction. Use only dimethylaminocinnamaldehyde (DMACA - Indole Spot Reagent, Cat. no. Z65) for indole testing.

This medium is non-selective so most UTI pathogens will grow. Colonies that are clear and do not react with the chromogenic substrates must be further tested with appropriate biochemical or serological tests for definitive identification. Fastidious organisms such as *Mycoplasma*, *Neisseria*, and *Haemophilus* cannot grow on this medium.

Minimize exposure of HardyCHROM™ UTI medium to light before and during incubation, as light can destroy the chromogens.

Refer to the keyword "Limitations", in the Hardy Diagnostics' software program HUGO™, for more information regarding general limitations on culture media.

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

The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Escherichia coli</i> ATCC® 25922	A	24hr	35°C	Aerobic	Growth; medium sized rose to magenta colonies with darker pink centers
<i>Klebsiella pneumoniae</i> ATCC® 13883	A	24hr	35°C	Aerobic	Growth; large, deep blue or dark indigo colonies

<i>Enterococcus faecalis</i> ATCC® 29212	A	24hr	35°C	Aerobic	Growth; small, teal to turquoise colonies
<i>Proteus mirabilis</i> ATCC® 12453	A	24hr	35°C	Aerobic	Growth; clear to light yellow colonies with golden-orange color diffused through surrounding media
<i>Staphylococcus aureus</i> ATCC® 25923	A	24hr	35°C	Aerobic	Growth; opaque, cream to white colored colonies
<i>Pseudomonas aeruginosa</i> ATCC® 27853	A	24hr	35°C	Aerobic	Growth; colorless to light yellow-green, translucent colonies, which may have a slight iridescence
<i>Staphylococcus saprophyticus</i> ATCC® 15305	A	24hr	35°C	Aerobic	Growth; opaque, pink colonies
<i>Candida albicans</i> ATCC® 10231	A	24hr	35°C	Aerobic	Growth; small, white, moist colonies
<i>Citrobacter freundii</i> ATCC® 8090	A	24hr	35°C	Aerobic	Growth; dark blue colonies, often with a rose halo in the surrounding media

* Refer to the keyword "Inoculation Procedures", in the Hardy Diagnostics' software program HUGO™, for a description of inoculation procedures.

USER QUALITY CONTROL

Check for signs of contamination and deterioration. Users of commercially prepared media may be required to perform quality control testing with at least one known organism to demonstrate growth or a positive reaction; and at least one organism to demonstrate inhibition or a negative reaction (where applicable). Refer to the following keywords, in the Hardy Diagnostics' software program HUGO™, for more information on QC: "Introduction to QC", "QC of Finished Product", and "The CLSI (NCCLS) Standard and Recommendations for User QC of Media". Also see listed references for more information. ⁽¹⁻⁵⁾

PHYSICAL APPEARANCE

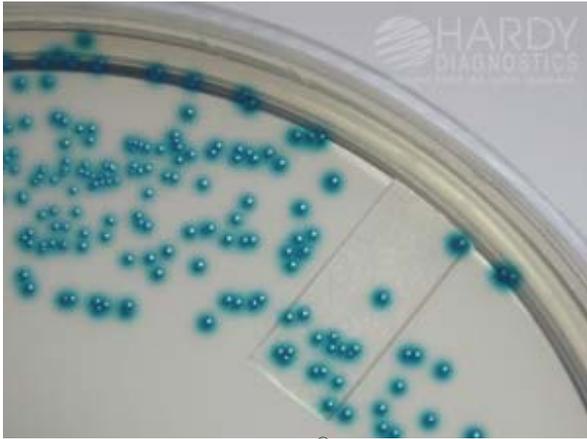
HardyCHROM™ UTI should appear translucent, and light off-white in color.



Escherichia coli (ATCC® 25922) colonies growing on HardyCHROM™ UTI (Cat. no. G313). Incubated aerobically for 24 hours at 35 deg. C.



Klebsiella pneumoniae (ATCC® 13883) colonies growing on HardyCHROM™ UTI (Cat. no. G313). Incubated aerobically for 24 hours at 35 deg. C.



Enterococcus faecalis (ATCC® 29212) colonies growing on HardyCHROM™ UTI (Cat. no. G313). Incubated aerobically for 24 hours at 35 deg. C.



Proteus mirabilis (ATCC® 12453) colonies growing on HardyCHROM™ UTI (Cat. no. G313). Incubated aerobically for 24 hours at 35 deg. C.



Staphylococcus aureus (ATCC® 25923) colonies growing on HardyCHROM™ UTI (Cat. no. G313). Incubated aerobically for 24 hours at 35 deg. C.



Pseudomonas aeruginosa (ATCC® 27853) colonies growing on HardyCHROM™ UTI (Cat. no. G313). Incubated aerobically for 24 hours at 35 deg. C.



Staphylococcus saprophyticus (ATCC® 15305) colonies growing on HardyCHROM™ UTI (Cat. no. G313). Incubated aerobically for 24 hours at 35 deg. C.



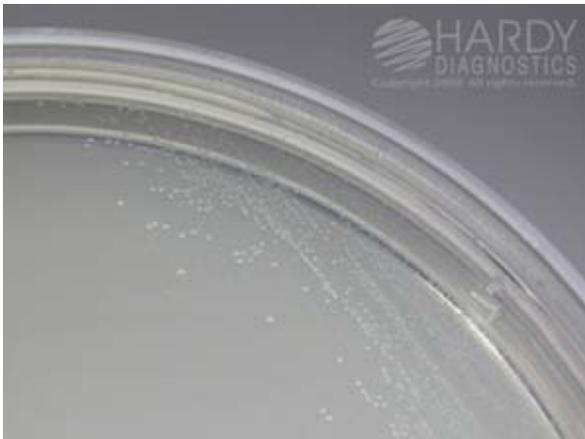
Candida albicans (ATCC® 10231) colonies growing on HardyCHROM™ UTI (Cat. no. G313). Incubated aerobically for 24 hours at 35 deg. C.



Citrobacter freundii (ATCC® 8090) colonies growing on HardyCHROM™ UTI (Cat. no. G313). Incubated aerobically for 24 hours at 35 deg. C.



Lactobacillus fermentum (ATCC® 9338), **left**, and *Lactobacillus acidophilus* (ATCC® 4356), **right**, colonies growing on HardyCHROM™ UTI (Cat. no. G313). Incubated aerobically for 24 hours at 35 deg. C.



Clinical strain of group B streptococci (GBS) growing on HardyCHROM™ UTI (Cat. no. G313). Incubated aerobically for 24 hours at 35 deg. C.



Clinical strains of group C streptococci growing on HardyCHROM™ UTI (Cat. no. G313). Incubated aerobically for 24 hours at 35 deg. C.



Streptococcus agalactiae (ATCC® 13813, non-hemolytic), **left**, and *Streptococcus agalactiae* (ATCC® 12386, hemolytic), **right**, colonies growing on HardyCHROM™ UTI (Cat. no. G313). Incubated aerobically for 24 hours at 35 deg. C.

REFERENCES

1. Anderson, N.L., et al. 2005. *Cumitech 3B; Quality Systems in Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.
2. Murray, P.R., et al. 2007. *Manual of Clinical Microbiology*, 9th ed. American Society for Microbiology, Washington, D.C.
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4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
5. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.
6. Merlino, J., et al. 1996. *Journal of Clinical Microbiology*, American Society for Microbiology; 35:1788-1793.

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

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HARDY DIAGNOSTICS

1430 West McCoy Lane, Santa Maria, CA 93455, USA

Phone: (805) 346-2766 ext. 5658

Fax: (805) 346-2760

Website: www.HardyDiagnostics.com

Email: TechService@HardyDiagnostics.com

Distribution Centers:

California · Washington · Utah · Arizona · Texas · Ohio · Florida

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