

# Instructions for Use

## HardyCHROM™ HUrBi™ Biplate

Cat. no. J100	HUrBi™, 15x100mm Biplate, 10ml/10ml	10 plates/bag
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### INTENDED USE

HardyCHROM™ HUrBi™ Biplate is a selective chromogenic medium recommended for the cultivation, differentiation and enumeration of various gram-negative and gram-positive bacteria, and yeast based on colony color and morphology. Selective agents have been added to the each side of the biplate to select for growth of gram-positive organisms and yeast on one side and to select for growth of gram-negative organisms on the other side of the biplate.

### SUMMARY

Originally HUrBi™ was formulated for the isolation and differentiation of urinary pathogens but the medium can be used in a variety of other applications to assist in the characterization of a select group of microorganisms. Chromogenic substrates (chromogens) incorporated into HUrBi™ produce different colored compounds when they are degraded by specific microbial enzymes. Thus HUrBi™ can be used for the cultivation and differentiation of different groups of organisms with only a minimum number of confirmatory tests. The original HardyCHROM™ UTI (Cat. no. G313) formula has been modified for use in a biplate format.

Peptones supply the necessary nutrients, and the mixture of chromogens permit detection and differentiation of the isolated organisms. Different selective agents have been added to each side of the biplate to select for growth of gram-positive organisms and yeast on one side and to select for growth of gram-negative organisms on the other side of the biplate. The swarming of *Proteus* is partially to completely inhibited.

### FORMULA

Ingredients per liter of deionized water:\*

Peptones	16.0gm
Chromogenic Mixture	5.0gm
Selective Agents	3.0gm
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.

Refer to the document "[Storage](#)" for more information.

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

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.<sup>(2-4)</sup> 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.<sup>(1-5)</sup>

**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.

### Urine specimens:

Inoculate both sides of the biplate as soon as possible after specimen collection. For quantitative testing streak each side of the plate with 0.01ml (Cat. no. HS10R) or 0.001ml calibrated loop (Cat. no. HS1R).

### Other specimens types:

Inoculate both sides of the biplate with a broth or other specimen type. Streak each side of the plate for isolation.

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 48 hours. Yeast may require 48 hours for adequate growth.

Do not incubate in an atmosphere supplemented with CO<sub>2</sub>.

## INTERPRETATION OF RESULTS

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

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.

Growth of gram-positive organisms and yeast will only occur on Side I of the biplate. Growth of gram-negative organisms will only occur on Side II.

### **Side I – For Gram Positive Bacteria and Yeast**

*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.

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

*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.

## Side II – For Gram Negative Bacteria



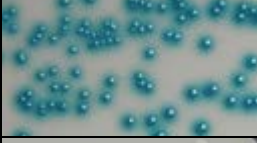

*Escherichia coli* produces medium to large sized colonies that are rose to magenta in color, with darker pink centers. No further testing is needed.


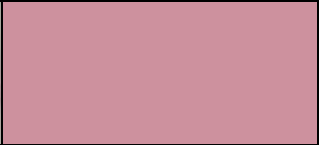
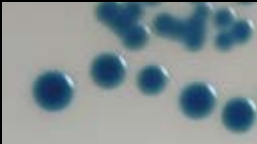







Colonies that resemble *E. coli* (pink to rose), but are small or pinpoint in size, require further identification procedures such as the Indole Spot Test (DMACA, Cat. no. Z65). See "Limitations" section below.

*Proteus*, *Morganella*, and *Providencia* spp. produce clear to light yellow colonies with golden-orange halo diffused through surrounding media. 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. H<sub>2</sub>S 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.

Organism	Description	Photo	Color
<i>Staphylococcus aureus</i>	opaque, cream to white colored colonies		
<i>Staphylococcus saprophyticus</i>	opaque, pink colonies		
<i>Enterococcus</i> spp.	teal to turquoise 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>E. coli</i>	rose to magenta colonies with darker pink centers		
<i>Klebsiella, Enterobacter, and Serratia</i> spp.	deep blue or dark indigo colonies		
<i>Citrobacter</i> spp.	dark blue colonies often with a rose halo in the surrounding media		
<i>Proteus, Morganella, and 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>Pseudomonas</i> spp.	colorless to light yellow-green colonies		

## LIMITATIONS

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

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.

Colonies that are clear and do not react with the chromogenic substrates must be tested further with appropriate biochemical or serological tests for definitive identification. Fastidious organisms such as *Mycoplasma*, *Neisseria*, and *Haemophilus* cannot grow on this medium.

*Enterococcus faecalis* growing as a teal colored film, on Side II, should be investigated as a possible vancomycin-resistant enterococci (VRE).

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

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

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

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Staphylococcus aureus</i> ATCC® 25923	A/B	24hr	35°C	Aerobic	<b>Side I:</b> Growth; opaque, cream to white colored colonies <b>Side II:</b> Inhibited
<i>Staphylococcus saprophyticus</i> ATCC® 15305	A/B	24hr	35°C	Aerobic	<b>Side I:</b> Growth; opaque, pink colonies <b>Side II:</b> Inhibited
<i>Enterococcus faecalis</i> ATCC® 29212	A/B	24hr	35°C	Aerobic	<b>Side I:</b> Growth; small, teal to turquoise colonies <b>Side II:</b> Inhibited
<i>Candida albicans</i> ATCC® 10231	A/B	24hr	35°C	Aerobic	<b>Side I:</b> Growth; small, white, moist colonies <b>Side II:</b> Inhibited
<i>Escherichia coli</i> ATCC® 25922	B/A	24hr	35°C	Aerobic	<b>Side I:</b> Inhibited <b>Side II:</b> Growth; medium sized rose to magenta colonies, with darker centers
<i>Klebsiella pneumoniae</i> ATCC® 13883	B/A	24hr	35°C	Aerobic	<b>Side I:</b> Inhibited <b>Side II:</b> Growth; large, deep blue or dark indigo colonies
<i>Proteus mirabilis</i> ATCC® 12453	B/A	24hr	35°C	Aerobic	<b>Side I:</b> Inhibited <b>Side II:</b> Growth; clear to light yellow colonies with golden-orange color diffused through surrounding media
<i>Pseudomonas aeruginosa</i> ATCC® 27853	B/A	24hr	35°C	Aerobic	<b>Side I:</b> Inhibited <b>Side II:</b> Growth; colorless to light yellow-green, translucent colonies, which may have a slight iridescence
<i>Citrobacter freundii</i> ATCC® 8090	B/A	24hr	35°C	Aerobic	<b>Side I:</b> Inhibited <b>Side II:</b> Growth; dark blue colonies, often with a rose halo in the surrounding media

\***Side I/Side II** Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

## USER QUALITY CONTROL

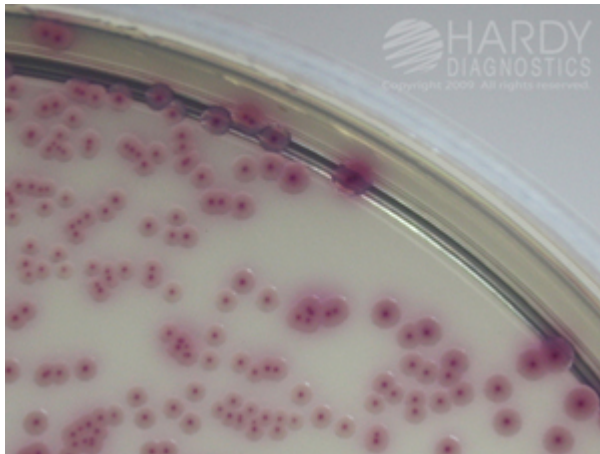
Check for signs of contamination and deterioration. Users of commercially prepared media may be required to perform quality control testing with at least one known organism to demonstrate growth or a positive reaction; and at least one organism to demonstrate inhibition or a negative reaction (where applicable). See listed references for more information.  
(1-5)

## PHYSICAL APPEARANCE

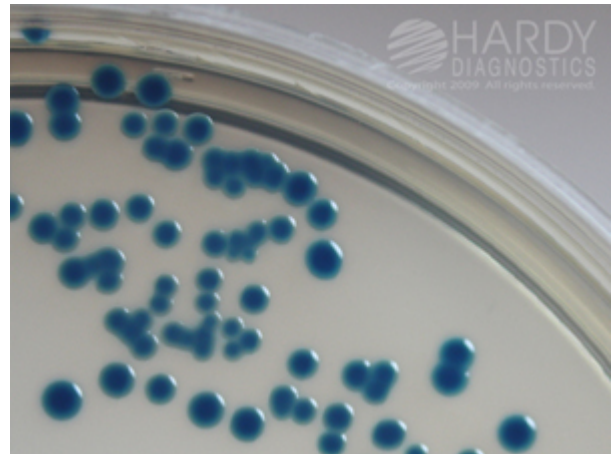
HardyCHROM™ Urine Biplate (HUrBi™) should appear as follows:

HUrBi™ POS (Side I) should appear translucent, and light off-white in color.

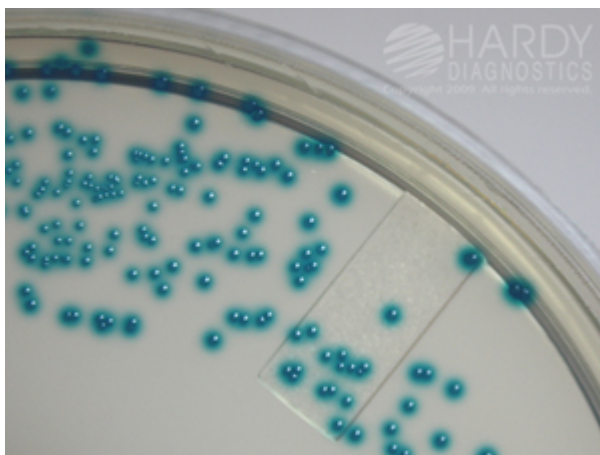
HUrBi™ NEG (Side II) should appear translucent, and light amber in color; may have a fine precipitate.



*Escherichia coli* (ATCC® 25922) colonies growing on HUrBi™, Side II (Cat. no. J100). Incubated aerobically for 24 hours at 35 deg. C.



*Klebsiella pneumoniae* (ATCC® 13883) colonies growing on HUrBi™, Side II (Cat. no. J100). Incubated aerobically for 24 hours at 35 deg. C.



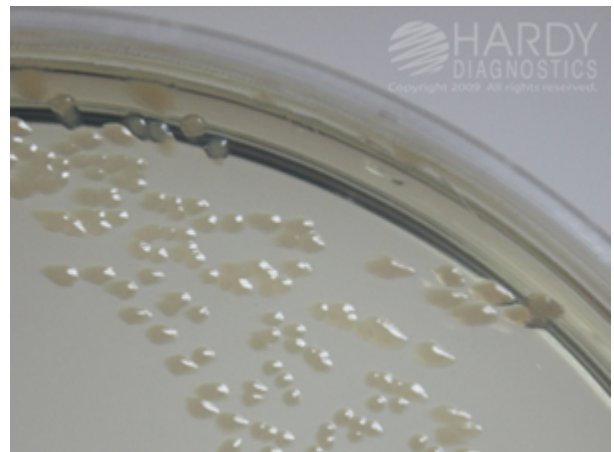
*Enterococcus faecalis* (ATCC® 29212) colonies growing on HUrBi™, Side I (Cat. no. J100). Incubated aerobically for 24 hours at 35 deg. C.



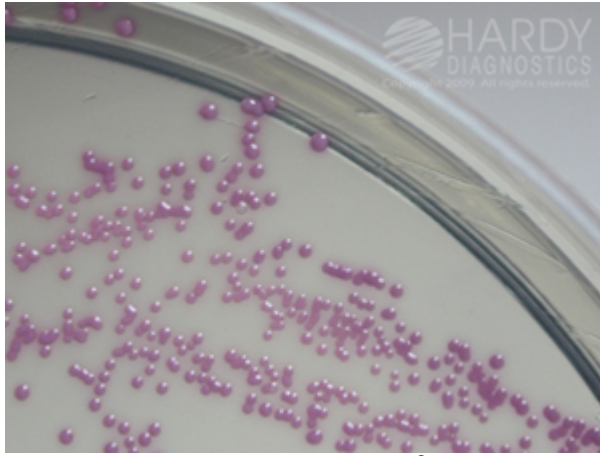
*Proteus mirabilis* (ATCC® 12453) colonies growing on HUrBi™, Side II (Cat. no. J100). Incubated aerobically for 24 hours at 35 deg. C.



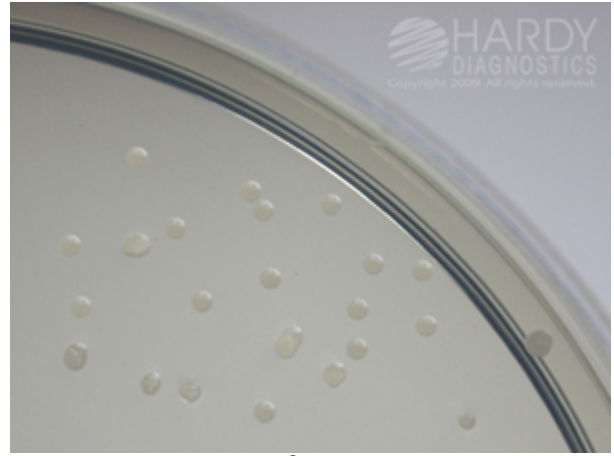
*Staphylococcus aureus* (ATCC® 25923) colonies growing on HUrBi™, Side I (Cat. no. J100). Incubated aerobically for 24 hours at 35 deg. C.



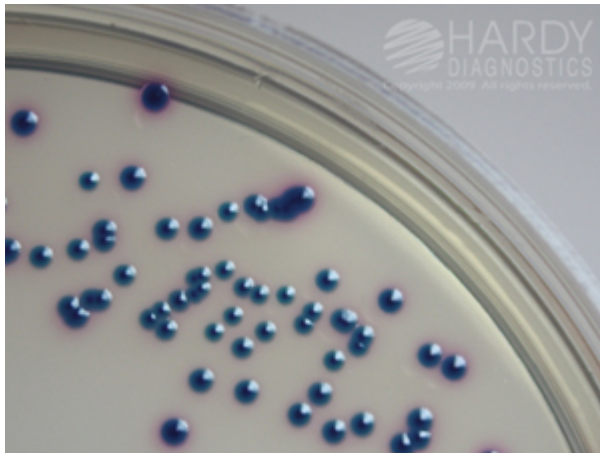
*Pseudomonas aeruginosa* (ATCC® 27853) colonies growing on HUrBi™, Side II (Cat. no. J100). Incubated aerobically for 24 hours at 35 deg. C.



*Staphylococcus saprophyticus* (ATCC® 15305) colonies growing on HUrBi™, Side I (Cat. no. J100). Incubated aerobically for 24 hours at 35 deg. C.



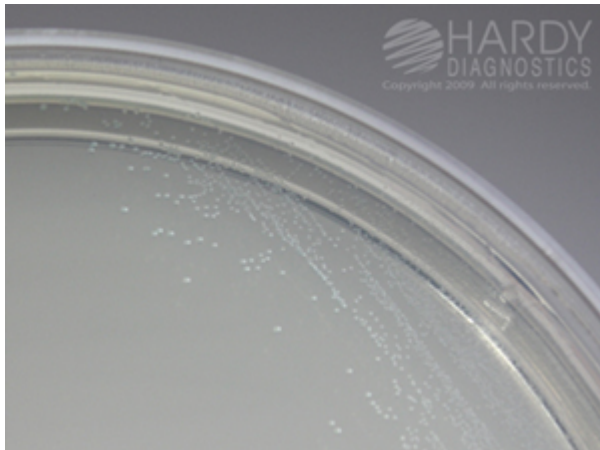
*Candida albicans* (ATCC® 10231) colonies growing on HUrBi™, Side I (Cat. no. J100). Incubated aerobically for 24 hours at 35 deg. C.



*Citrobacter freundii* (ATCC® 8090) colonies growing on HUrBi™, Side II (Cat. no. J100). Incubated aerobically for 24 hours at 35 deg. C.



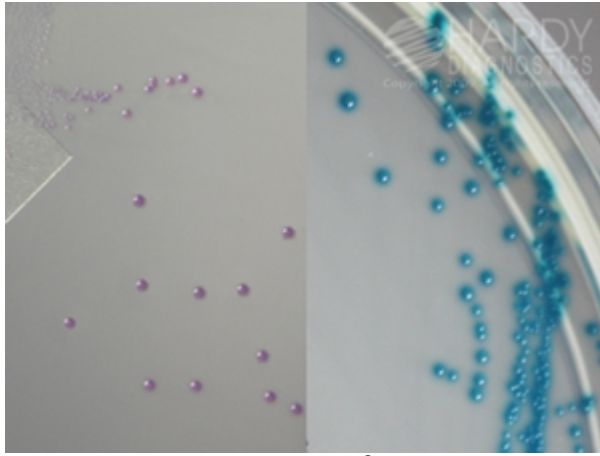
*Lactobacillus fermentum* (ATCC® 9338), **left**, and *Lactobacillus acidophilus* (ATCC® 4356), **right**, colonies growing on HUrBi™, Side I (Cat. no. J100). Incubated aerobically for 24 hours at 35 deg. C.



Clinical strain of group B streptococci (GBS) growing on HUrBi™, Side I (Cat. no. J100). Incubated aerobically for 24 hours at 35 deg. C.



Clinical strains of group C streptococci growing on HUrBi™, Side I (Cat. no. J100). 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 HURBi™, Side I (Cat. no. J100). 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.
3. Forbes, B.A., et al. 2007. *Bailey and Scott's Diagnostic Microbiology*, 12th ed. C.V. Mosby Company, St. Louis, MO.
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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