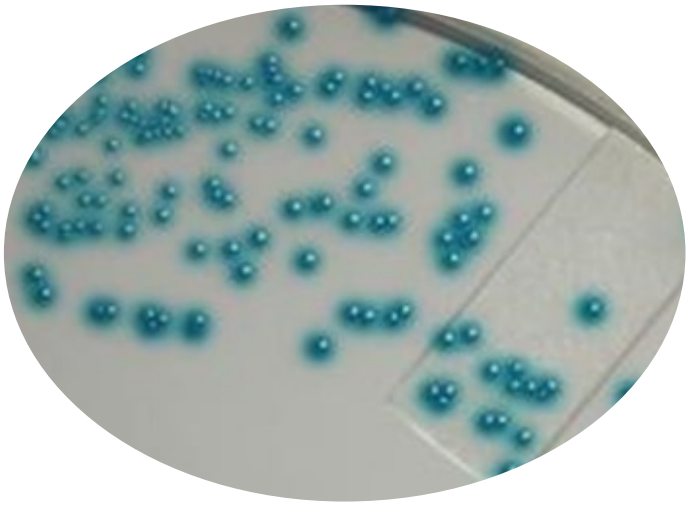
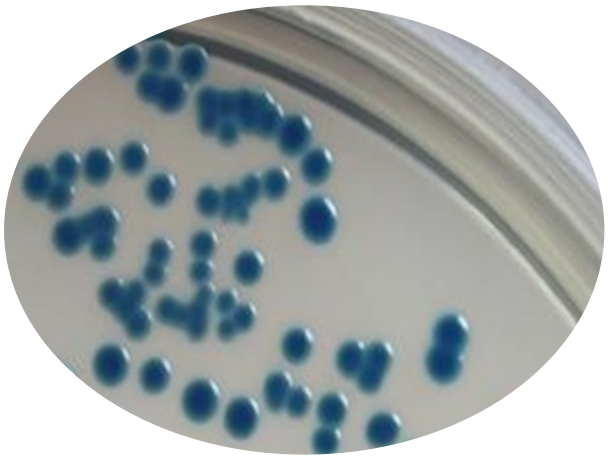
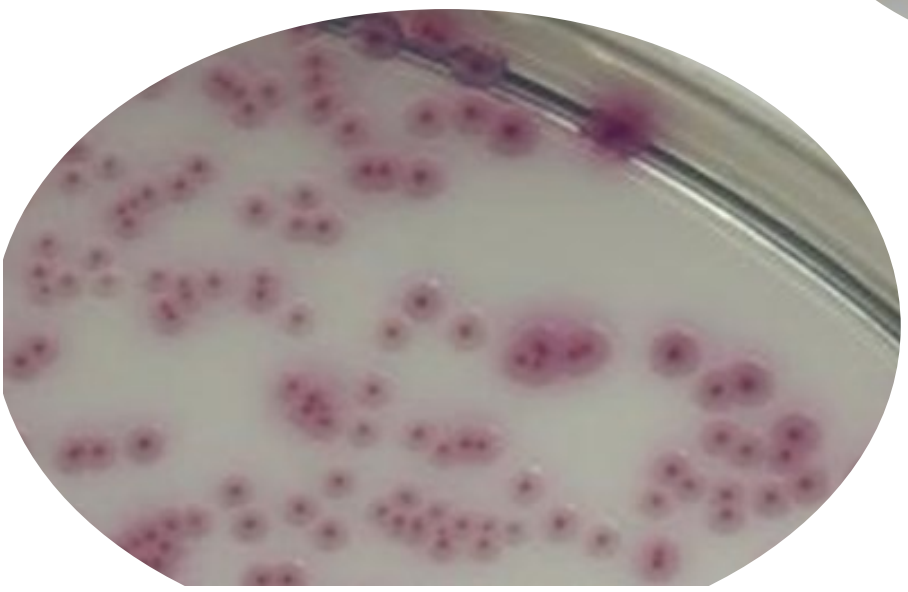
MicroVet Diagnostics

Color-Spot

Chromogenic Microbiology Culture Identification & Sensitivity System







Users Guide

Contents

MicroVet Diagnostics

Contact Information

800-640-7780

864-382-3131 (fax)

admin@VetDiagnosticSolutions.com

Your Account Number

_____

Your Account Rep_____

Account Rep Phone number_____

Your Technical Support Specialist_____

Tech Support Direct dial number_____

To Order Supplies Visit

www.MicroVetDiagnostics.com

Materials Required

Item	Order Number
Color-Spot Chrome Agar (bi-plate)	M200
Muller Hinton Agar	M201
37 deg. Incubator	M211
Acrylic Tip applicator swabs	20216
Inoculating loops	20217
Sensitivity Dilution Solution	20235
AST sensitivity rings*	20219-20243
Select sensitivity disks**	custom
Disk Dispenser	M202
Color Chart	included and available on line at www.MicroVetdiagnostics.com
Metric Ruler or measuring guide	Included
Sensitivity zone interpretation chart	Included

- Only needed if using the AST ring sensitivity method

** Only needed if using the customized sensitivity method

MicroVet Diagnostics

Color-Spot

Chrom-Agar Bacterial Identification Procedure

MicroVet Color Spot Biplate

INTENDED USE

MicrVet Color Spot 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 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 formulated for the isolation and differentiation of urinary pathogens, the medium can be use in a variety of other applications to assist in the characterization of a select group of microorganisms. Chromogenic substrates (chromogens) incorporated into the media produce different colored compounds when they are degraded by specific microbial enzymes. Thus the media can be used for the cultivation and differentiation of different groups of organisms from sources such as ears, skin and wounds, uterine and many other sources.

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.

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, or cracking), contamination, or if the expiration date has passed. Contact your technical support specialist with any issues.

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.

PRECAUTIONS

This product is for *in vitro* diagnostic use only and is to be used only by adequately trained and qualified 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*

PROCEDURE - Organism Identification

Culture 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.

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:

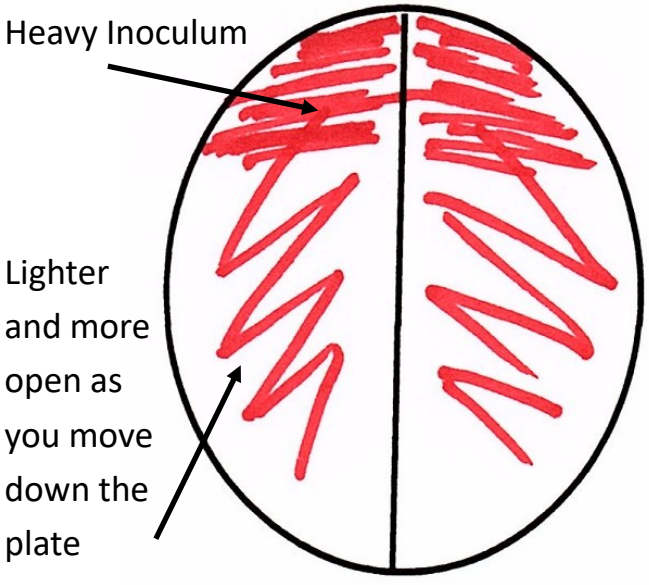
Inoculate both sides of the biplate as soon as possible after specimen collection. For quantitative testing streak each side of the plate with 0.01ml calibrated loop.

Other specimens types:

Inoculate both sides of the biplate with the specimen. Streak each side of the plate for isolation.

Incubate plates in an inverted position, aerobically at 37 +/- 2 degrees C. for 18 to 24 hours. Examine plates for colonies showing typical morphology and color after 24 hours, but no later than 72 hours. Yeast may require 48 hours for adequate growth.

Do not incubate in an atmosphere supplemented with CO_2.



Streaking For Isolation

Streaking for isolation is where a heavy amount of the specimen is introduced to the top of the plate (on both sides) then starting in the heavy inoculum , zig zag down the plate making each zig and zag more open than the last. This dilutes the sample as you move down the plate and will allow for well defined and well isolated colonies of bacteria. This will make distinguishing each organism much easier.

INTERPRETATION OF RESULTS

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

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/ pseud-intermedius produce opaque, cream to white colored colonies. **Note:** Colonies may turn pink after 72 hours.

Staphylococcus saprophyticus produce opaque, pink colonies.

Staphylococcus epidermidis grows as small, white colonies.

Enterococcus spp. appear as small, teal to turquoise colored colonies.

Candida albicans, *Candida tropicalis*, and *Candida glabrata* produce small, opaque, white, moist colonies.

Candida krusei appears as small, white, dry colonies which have a rough appearance.

Listeria monocytogenes or other *Listeria* spp. may be present in urine. Colonies of *Listeria* are very small, blue to bluegreen colonies. Perform a Gram stain of organisms producing small, blue to blue-green colonies. The presence of gram-positive bacilli is suggestive of *Listeria* spp.

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.

See Color and Morphology Chart on next pages

Side II – For Gram Negative Bacteria

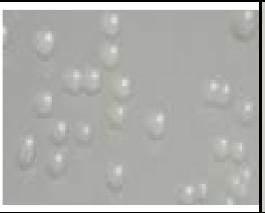
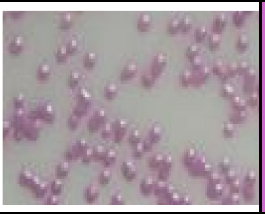
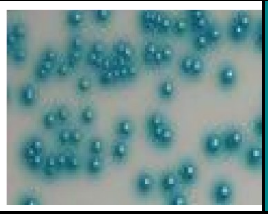
Escherichia coli produces medium to large sized colonies that are rose to magenta in color, with darker pink centers.

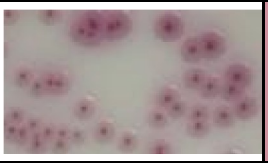
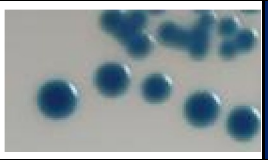
Proteus, *Morganella*, and *Providencia* spp. produce clear to light yellow colonies with golden-orange halo diffused through surrounding media. Approximately 50% of *Proteus vulgaris* isolates will produce blue-green or green colonies with a golden-orange halo.

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.

Pseudomonas spp. produce colorless to light yellow-green, translucent colonies which may have a slight iridescence with crinkled edges.

Organism	Description	Photo	Color
Staphylococcus aureus	opaque, cream to white colored colonies		
Staphylococcus saprophyticus	opaque, pink colonies		
Enterococcus spp.	teal to turquoise colonies		

Organism	Description	Photo	Color
Candida albicans, *Candida krusei*, *Candida tropicalis*, and *Candida glabrata*	small, opaque, white, moist colonies (*C. krusei* will be a rough colony)		
E. coli	rose to magenta colonies with darker pink centers		
Klebsiella, *Enterobacter*, and *Serratia* spp.	deep blue or dark indigo colonies		
Citrobacter spp.	dark blue colonies often with a rose halo in the surrounding media		
Proteus, *Morganella*, and *Providencia* spp.	clear to light yellow colonies with goldenorange halo in the surrounding media (some *Proteus vulgaris* colonies will be blue-green)		
Pseudomonas spp.	Colorless to light yellowgreen 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 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).

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 vancomycinresistant enterococci (VRE).

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

QUALITY CONTROL

The following organisms are routinely used for testing at MicroVet

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

USER QUALITY CONTROL

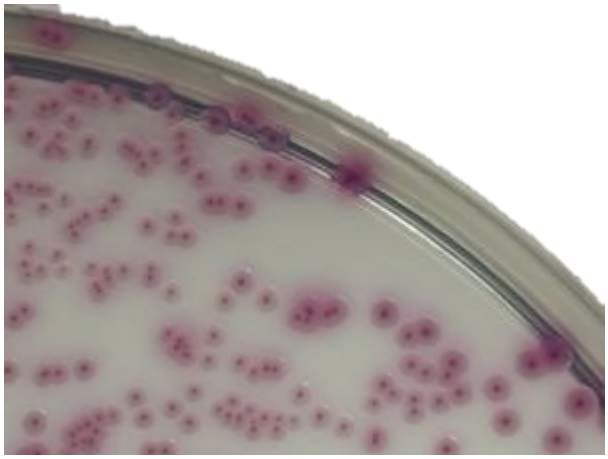
Check for signs of contamination and deterioration. Users of commercially prepared media may be required to perform quality control testing with at least one known organism to demonstrate growth or a positive reaction; and at least one organism to demonstrate

PHYSICAL APPEARANCE

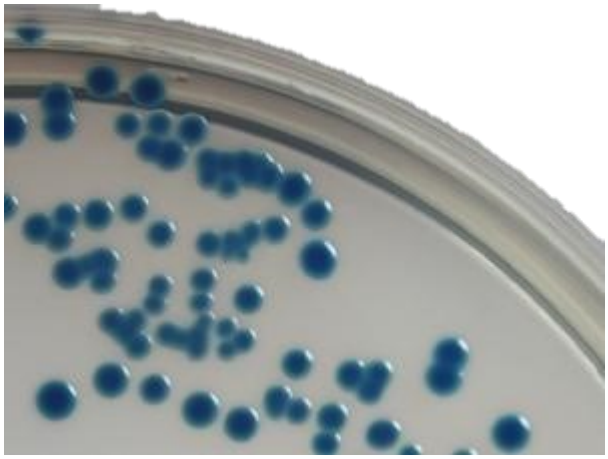
Biplate (HUrBi™) should appear as follows:

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

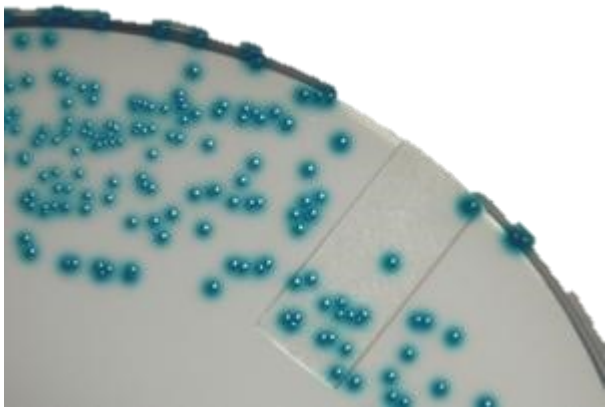
NEG (Side II) should appear translucent, and light amber in color; may have a fine precipitate.



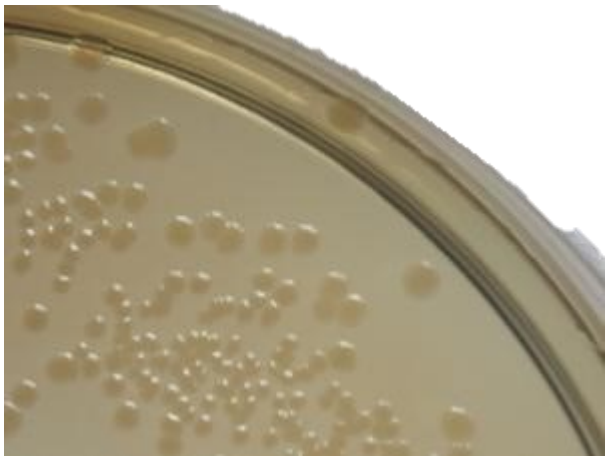
Escherichia coli (ATCC® 25922) colonies growing on Side II Incubated aerobically for 24 hours at 35 deg. C.



Klebsiella pneumoniae (ATCC® 13883) colonies growing on Side II incubated aerobically for 24 hours at 35 deg. C.

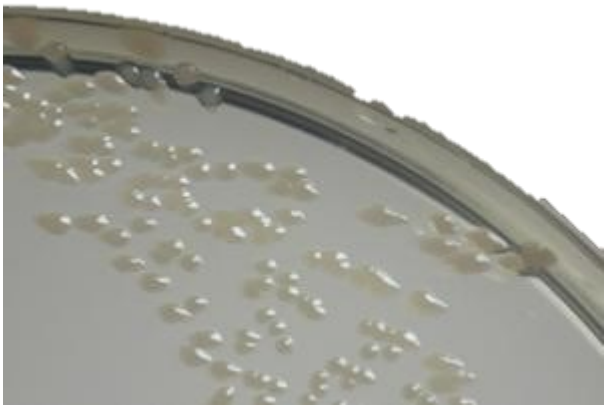


Enterococcus faecalis (ATCC® 29212) colonies growing on Side I Incubated aerobically for 24 hours at 35 deg. C.

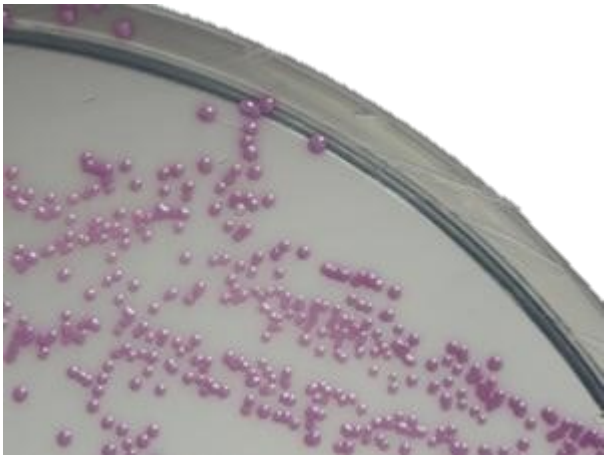


Proteus mirabilis (ATCC® 12453) colonies growing on Side II Incubated aerobically for 24 hours at 35 deg. C.

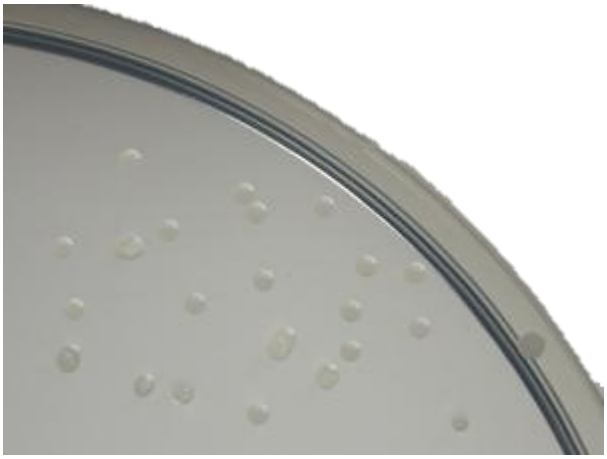
Staphylococcus aureus (ATCC® 25923) colonies growing on Side I Incubated aerobically for 24 hours at 35 deg. C.



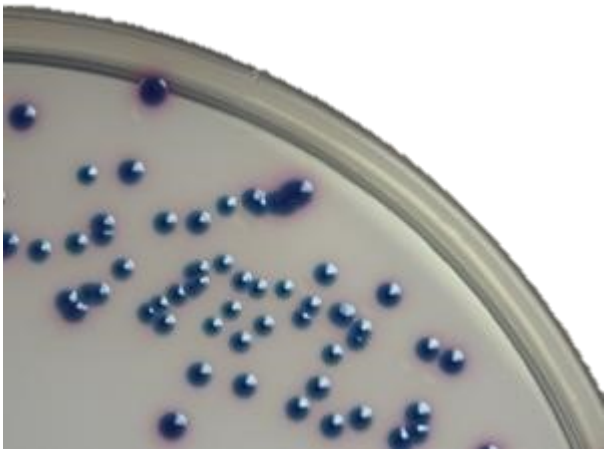
Pseudomonas aeruginosa (ATCC® 27853) colonies growing on Side II Incubated aerobically for 24 hours at 35 deg. C.



Staphylococcus saprophyticus (ATCC® 15305) colonies growing on Side I . Incubated aerobically for 24 hours at 35 deg. C.

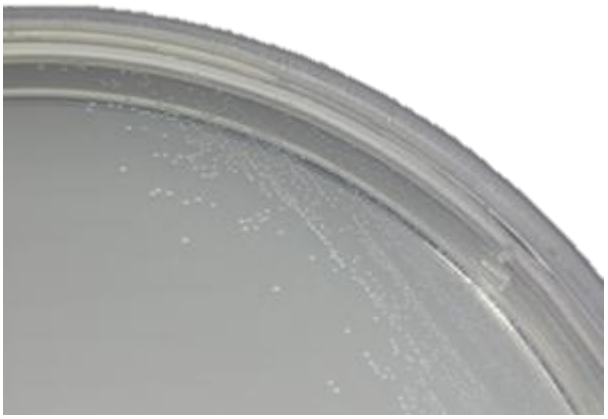


Candida albicans (ATCC® 10231) colonies growing on Side I Incubated aerobically for 24 hours at 35 deg. C.

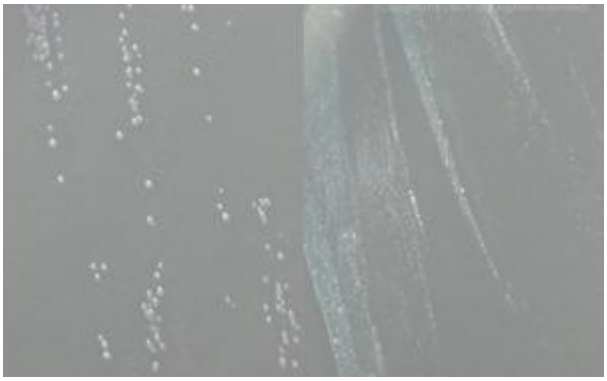


Citrobacter freundii (ATCC® 8090) colonies growing Side II Incubated aerobically for 24 hours at 35 deg. C.

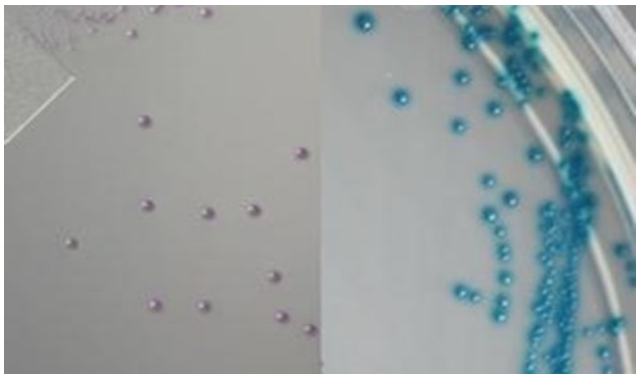
Lactobacillus fermentum (ATCC® 9338), **left**, and *Lactobacillus acidophilus* (ATCC® 4356), **right**, colonies growing on Side I Incubated aerobically for 24 hours at 35 deg. C.



Clinical strain of group B streptococci (GBS) growing on Side I Incubated
aerobically for 24 hours at 35 deg. C.



Clinical strains of group C streptococci growing on Side I Incubated aerobically
for 24 hours at 35 deg. C.



Streptococcus agalactiae (ATCChemolytic), **left**, and *Streptococcus agalactiae* (ATCC® 12386, hemolytic), **right**, colonies growing on Side I Incubated aerobically for 24 hours at 35 deg. C.

PROCEDURE - Antibiotic Sensitivity

Supplies Needed:

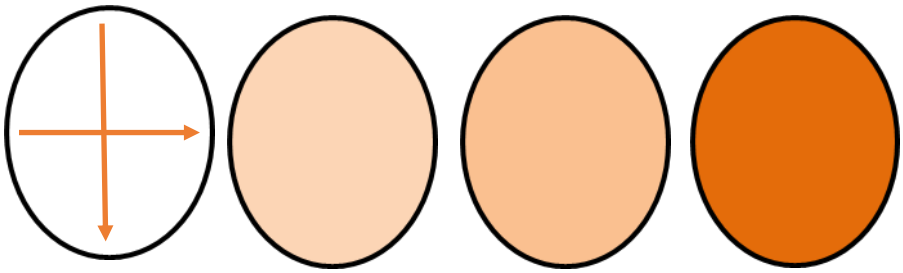
- Muller Hinton Sensitivity media (at room temperature)
- Sensitivity Dilution Solution
- Swab (acrylic tipped applicator)
- Turbidity Standard
- Selected antibiotic sensitivity disks and dispenser
- Incubator
- Metric ruler
- Report Chart

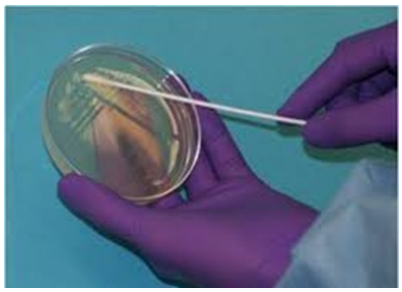
Allow the Muller Hinton sensitivity plates to warm to room temperature. The agar surface should be to dry prior to inoculating.

DO NOT transfer the organism directly from the ID plate to the Muller Hinton Sensitivity plate.

1. Using the acrylic tipped applicator, "scoop up" 3 to 4 well isolated colonies of the organism you have grown on the ID plate.
2. Insert that swab into the dilution solution tube and swirl it around suspending the bacteria in the solution.
3. Compare the turbidity of the bacterial solution to the turbidity standard chart that is included in your kit. The dilution tube should be close (+/-) to the turbidity in the standard depicted in the image. If your solution is not turbid enough, collect another colony with the swab until you achieve close to the desired turbidity. If your solution is overly turbid, you can add drops of the solution from a fresh un-used dilution tube until the dilution lightens to the desired turbidity.
4. Using the same swab, inoculate the Muller Hinton Sensitivity plate by striking a line with the swab from top to bottom and from side to side. Then using that swab, completely cover the surface of the media with the bacterial dilution. The goal here is to completely "paint" the surface of the media with the solution. It is best to cover the

4. Surface of the media in 3 plains. Top to bottom—side to side, then give the plate a quarter turn and cover from top to bottom—side to side then give the plate another quarter turn and repeat. This will ensure you have completely covered the surface of the media. NOTE: do not dip the swab into the solution each time, the initial "dip" of the swab into the solution is sufficient to properly inoculate the plate.



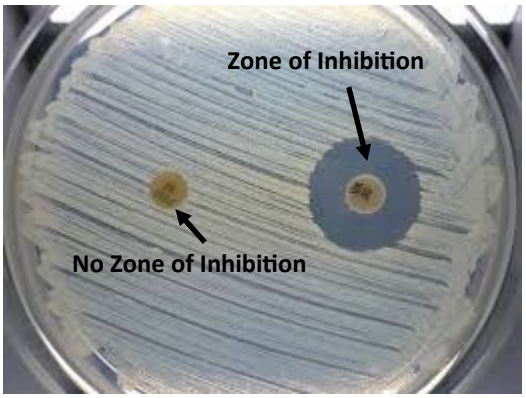


If using the customized disk dispenser method:

5. Load the antibiotic disk dispenser with your selected antibiotics. Your account representative can help you customize your antibiotic choices.
6. Place the disk dispenser over the inoculated Muller Hinton plate and press the plunger. This will dispense and tamp the antibiotic disks into place.
7. Put the cover on the Muller Hinton plate, label the plate with the name and date.
8. Incubate the plate by putting it in the incubator in an inverted position (the disks should be suspended upside down). For 18-24 hours but not longer than 36 hours.

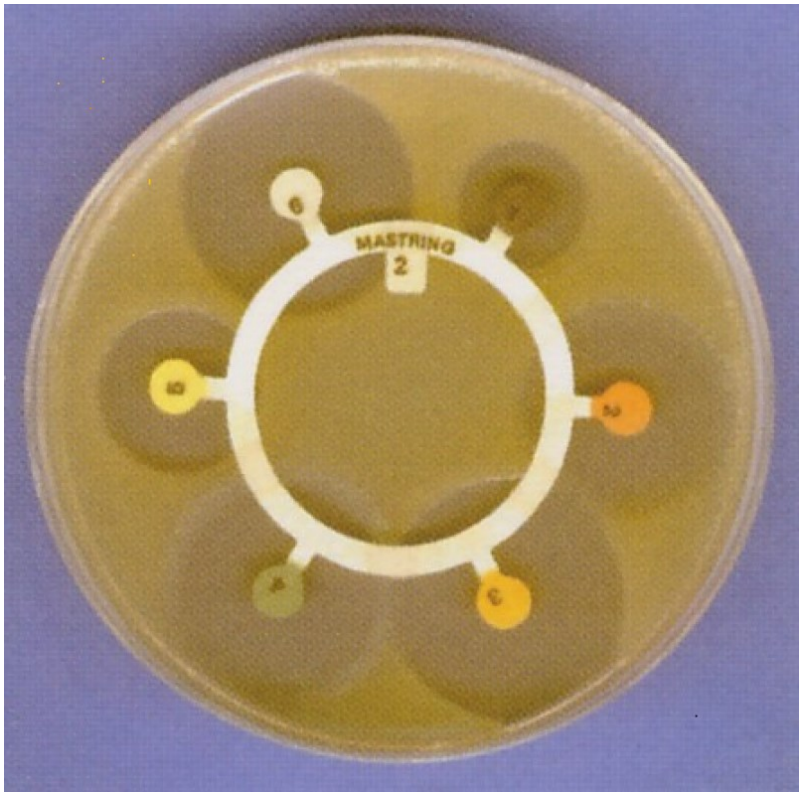
INTERPRETATION OF RESULTS

After incubation for 18-24 hours, the Muller Hinton plate should show growth over the entire surface of the media except where the antibiotics have inhibited growth. Note that in some instances there may not be a complete coverage of the media with bacterial growth rather speckled growth of isolated colonies, this is ok, the results can still be interpreted from this plate.

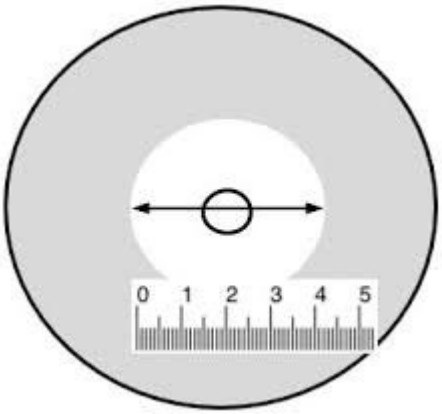


<u>If using the AST sensitivity "ring" system</u>

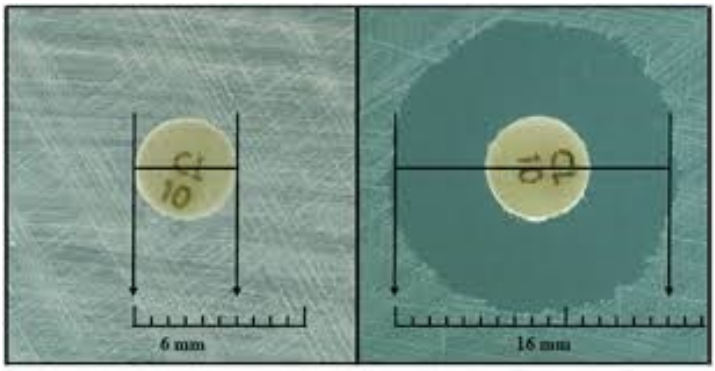
1. Select the appropriate AST Ring and bring to room temperature
2. Inoculate the plate as in steps 1-4 on page 16 & 17
3. Remove the selected AST ring from the foil pouch with gloved fingers or tweezers grabbing the inner portion of the ring.
4. Place the ring in the center of the inoculated muller hinton plate FACE DOWN and lightly tap in place.
5. Put the cover on the Muller Hinton plate, label the plate with the name and date.
6. Incubate the plate by putting it in the incubator in an inverted position (the disks should be suspended upside down). For 18-24 hours but not longer than 36 hours.
7. Interpret results as described on page 19



1. Reading from the underside of the plate (do not remove the cover) you will measure the **<u>diameter</u>** of the zone of inhibition (zone of no growth) around each of the antibiotic disks.
2. Using a metric ruler, measure across the zone of inhibition directly through the center of the disk. Note the drawing.

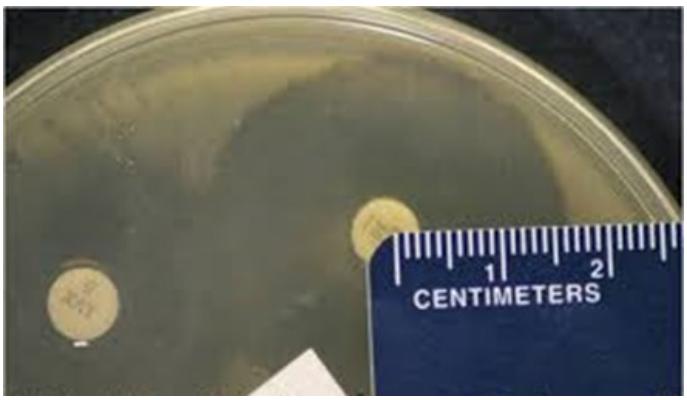


3. If there is no zone of inhibition, in other words if the bacteria grows right up to the antibiotic disk, this is a "0" or no zone result and is a "Resistant" antibiotic."
4. On the work sheet provided for you, log in the zone sizes for each antibiotic. Each antibiotic has a zone size on the work sheet that will indicate if the antibiotic is Sensitive, Resistant or Intermediate Resistant to that antibiotic.



NOTE: If you do not get solid growth over the surface of the media, but rather get a speckled looking growth, the plate is still useable. To interpret these results:

1. Using the same metric ruler, measure from the center of the antibiotic disk to the bacterial colony that is growing closest to the disk.
2. Since you are now measuring the radius, you will need to multiply that measurement by 2 to get the Diameter. So for example if your measurement was 15mm then your zone of inhibition would be 30mm. This is the number you would log on your result sheet for interpretation.
3. Note in the picture that there is one side of the zone missing, so the measurement goes from the disk to the one edge to get the radius. This result will be multiplied by 2 to get the Diameter.



Things to Note

1. The Chrome agar is sensitive to change in pH. Significant pH change will cause there to be a diffuse pinkish color appear throughout the media. The Media is still useable, however, this color change can make interpreting results more difficult. Note that the organism will grow on the surface of the media and not "in" the media, so it is easy to determine if the color is from bacteria or the pH change.
    A. Things that can cause the pH change:
        1. Storing the Media to Cold.
        2. Introduction of a urine with an extremely low or high pH.
2. Excessive exposure to light can affect to medias ability to properly ignite the proper color change. Try and protect the media from light especially when taken out to come to room temperature.
3. Most all bacteria will grow on this media, however, not all bacterial will ignite a specific color change that is within the identifiable color palate. Should you get growth that does not fit into the color palate, you should consider that this could be a unique pathogen and should be further investigated. This is where your reference laboratory comes in. Swab the unknown bacteria with a transport swab and submit to your reference laboratory.
4. **MRSA,** Note that if you get a Staphylococcus aureus or Intermedius, and suspect or are concerned about MRSA, using an Oxacillin disk as one of your antibiotic choices will let you know the Methicillin resistance status of the organism.
5. Disposal of your used plates. Each state has different rules regarding this type of waste disposal in a veterinary practice. We suggest you consider the growing of bacteria as a potential infectious agent and dispose of accordingly. If you choose to autoclave the plates, note that the media will liquify, so we suggest you put the plates in a sealable bag before autoclaving to avoid any messy clean-up. Running hot water with bleach over the media will also dissolve the media and sterilize the plates.
6. Be sure to use the acrylic tipped applicators in any bacterial setting as natural cotton tipped applicators are bleached in the manufacturing process and this can interfere with the ability to recover bacterial pathogens.