4. Organisms may be harvested either from the blood agar or Spectrum agar sections of the plate. Do not push wand into agar or drag across surface. If colonies are smaller than 1 mm, touch ten instead of five. For very small pinpoint colonies use an alternative method or perform a direct sensitivity.
5. Remove the Prompt® inoculation tube from the rack while still holding the inoculation wand and snap off the cap by bending it sideways.
6. Carefully insert the inoculation wand into the tube. Use a twisting motion to press down on the wand to form a tight seal.
7. To release the bacteria from the tip of the wand, vortex (if available) for 10 seconds. If a vortex is not available, vigorously shake and tap the inoculation tube until all bacteria have been rinsed from the tip. If necessary, allow tube to stand for 5 minutes and repeat mixing.
8. Remove wand from tube and discard. Follow steps 1-13 in the preceding section to inoculate the Mueller Hinton plate with the standardized suspension.

Note: The presence of more than one colony type or color on the Spectrum Quad plate is indicative of a mixed infection or possible contamination. If the former, a separate inoculum should be prepared for each one.

Limitations of the Procedure: The following factors may affect organism growth, colony color, and antibiotic sensitivity results:

- Improper specimen collection, storage, and inoculation.
- Initiation of antimicrobial therapy prior to inoculation.
- Improper incubation temperature and duration.
- Improper handling and storage of media prior to inoculation.
- Testing of mixed organisms.

Packaging: The Spectrum CS[tm] kit contains 5 each of individually wrapped Spectrum Quad and Mueller Hinton plates, 5 BBL Prompt® dilution sets, and accessories.
Reorder Product No. MCR-PLTSP500.

Spectrum™, Spectrum-Plus™, Spectrum-MS™, and Spectrum-CS™ culture systems are manufactured and distributed exclusively by:

Vetlab Supply
Palmetto Bay, FL 33157
(800)330-1522
(305)232-8421 Fax
www.vetlab.com

Rev. 2/2020

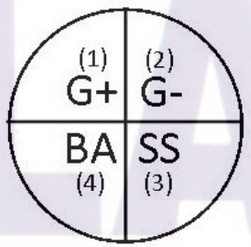
Spectrum CS™/IV Culture System

Product Information and Instructions

Intended Use
Spectrum CS™ is a complete in-house system that can provide a presumptive identification and antibiotic sensitivity for many bacterial organisms commonly known to cause disease in animals. The Spectrum CS system is intended for veterinary use only.

Product Features
The Spectrum CS culture system consists of a four chambered plate containing two selective chromogenic agars, selective Staphylococcus agar, and non-selective Tryptic Soy Agar with 5% sheep blood. A separate Mueller Hinton plate for performing antibiotic sensitivity testing via the Kirby-Bauer disk diffusion method is also provided. This unique combination provides a comprehensive scheme for identifying infectious agents and for determining the optimal antimicrobial(s) for treatment.

Spectrum [tm] chromogenic agars have been formulated to produce uniquely pigmented colonies when inoculated with those organisms for which the product has been validated. Each organism can then be visually differentiated on the basis of color and colony morphology. Depending upon the organism, color reactions may be either genus or species-specific. The Spectrum quad plate utilizes two selective agars that further classify organisms as either Gram positive (Section 1) or Gram negative (Section 2) .



A special selective agar (Section 3) aids in isolation and identification of Staphylococci. Staphylococcus aureus typically produces black colonies surrounded by a yellow zone. Other Staphylococci may produce black colonies with no color change. While Selective Staphylococcus media is markedly inhibitory to the growth of other Gram positive organisms, partial inhibition may occur with certain Gram negative organisms such as E. coli, Proteus, or Enterococcus but with no color change to the media.

Tryptic Soy agar with 5% Sheep blood (Section 4), commonly referred to as “blood agar”, is a non-selective medium that supports the growth of a wide range of bacteria. When grown on blood agar, certain organisms will elaborate hemolysins that will lyse red blood cells within the medium creating a clear or green zone around each colony. While this reaction can aid in the identification of some organisms, hemolysis by itself should not be used as a confirmatory test.

Mueller Hinton is the media of choice for antibiotic sensitivity testing. The BBL Prompt® System facilitates preparation of standardized suspensions of bacteria for inoculation of the Mueller Hinton agar plate.

Storage and Shelf Life
Each Spectrum agar and each Mueller Hinton plate are individually wrapped for extended shelf life. Plates should be stored inverted at 2°-8° C (36°-46° F) and protected from light. Do not freeze. The BBL Prompt® vials and wands should be stored at room temperature.

Materials Provided
5 Spectrum CS Quad plates
5 Mueller Hinton plates
10 Disposable 10 ul calibrated inoculating loops
10 Sterile cotton swabs
5 BBL Prompt® dilution sets

Additional materials required
Incubator
Antibiotic Sensitivity discs
Antibiotic Sensitivity disc dispenser
Caliper or ruler divided in millimeters

Initial Culture and Organism Identification

Spectrum plates should be removed from the refrigerator and allowed to warm to room temperature prior to inoculation. The surface of the agar should be inspected for moisture that could affect growth. If present, decant excess moisture or allow to evaporate prior to use. Inoculate each plate using established aseptic technique and place in a 37° C incubator inverted (media on top.). At 18-24 hours, inspect the plate for bacterial growth and note the color and morphology of the resulting colonies. For accurate results, plates must be read within 24 hours as prolonged incubation can alter the characteristic color reaction(s).

Quantitative Bacterial Count (Urine Culture Only)

The colony count can support a diagnosis of urinary tract infection. Interpretation is based on inoculation with a standardized 10 ul volume of specimen using the provided loop. Dip the loop into the specimen and streak one quadrant. Repeat for remaining quadrants dipping the loop each time. Following incubation, perform the colony count using only the quadrant with the greatest number of colonies. After counting, multiply the number of colonies by 100. As a rule, colony counts from cystocentesis samples ≥1000 CFU/ml (colony forming units/ml) generally support a diagnosis of urinary tract infection. Colony counts of 100-1000 CFU/ml should be viewed as suspicious. For samples collected via catheter in cats and male dogs, counts ≥10,000 cfu/ml are considered significant and for female dogs ≥100,000 cfu/ml. The appearance of a single colony on any quadrant is likely a contaminant.

Interpretation

Refer to the following table, provided color chart, and footnotes. The images and descriptions were obtained using pure cultures of the most commonly isolated subspecies of each organism. Some less common subspecies may yield varied color reactions. Mixed cultures should be carefully interpreted. See notes following table for suggested ancillary testing methods that may further aid in identification. Presumptive and/or questionable results should be verified using traditional culture methods and/or sent to a qualified reference laboratory.

Organism	(1) Spectrum Gram +	(2) Spectrum Gram -	(3) Staph. Select	(4) TSA w/5% Blood	Catalase[1]	Oxidase[2]
Group B Streptococcus	Light blue pinpoint colonies.	No Growth	Some species may produce small black colonies with no color change to media.	Pinpoint semi-transparent colonies with clear zone of beta hemolysis. Some species non-hemolytic. V[3]	Neg	NA
Enterococcus Spp.	Blue to turquoise pinpoint colonies.	No Growth	Generally no growth. Some species may produce small black colonies. No color change to media.	Pinpoint to small smooth colonies. Generally non-hemolytic.	Neg	Neg
E. coli	No Growth	Medium to large pink to red colonies.	Generally no growth. Some rare species may produce a few black colonies with no color change to media.	Medium size gray colonies with characteristic odor. Most species are non-hemolytic. V[3]	Pos	Neg
Staphylococcus aureus	Mauve to white colonies. Some species may appear pale yellow. V[3]	No Growth	Black colonies surrounded by yellow zone. See notes regarding other species of Staphylococcus.[5]	Medium-sized white to gray raised glistening colonies. Clear zone of (beta) hemolysis.	Pos	NA
Proteus mirabilis	No Growth	Clear to slightly orange colonies surrounded by brown pigment diffusing into media.	Some species may produce small black colonies with no color change to media.	Gray mucoid growth swarming over plate. Distinct colonies are rarely seen. Brown pigment diffusing into media.	Pos	Neg
Enterobacter Spp.	No Growth	Large metallic blue colonies surrounded by pink halo.	No Growth	Large mucoid gray colonies. Non-hemolytic.	Pos	Neg
Klebsiella pneumoniae	No Growth	Medium size metallic blue mucoid colonies; may or may not be surrounded by pink zone. V[3]	May produce a few black pinpoint colonies with no color change to media.	Large mucoid gray colonies. Non-hemolytic.	Pos	Neg
Pseudomonas aeruginosa	No Growth	Transparent slightly greenish colonies w/some diffusion of pyocyanin into media. Some species reddish brown.	No Growth	Medium size gray or bluish colonies with some coalescing.	Pos	Pos
Candida albicans	Medium to large mauve colonies. Some species may produce white colonies. V[3]	Small to medium off-white colonies	Medium to large gray mucoid colonies. No color change to medium.	Moist, opaque white to gray medium to large colonies.[4]	NA	NA

Footnotes

[1] The Catalase test using 3% hydrogen peroxide can aid in differentiating Staphylococcus from Streptococcus species.
[2] The Oxidase test can aid in differentiating Pseudomonas aeruginosa from other Gram negative bacteria and some Staphylococcus species. Oxyswab® (Prod. #MCR-GL60500) affords a simple method for performing this test.
V[3] indicates the potential for variability in color and growth characteristics of certain subspecies.
[4] Candida albicans and other yeasts may grow on all quadrants. Gross morphology and the presence of large blue to purple budding cells on Gram stain can help to differentiate yeast from bacteria.
[5] S. saprophyticus and S. epidermidis are considered non-pathogens that will form dark gray colonies without yellow zones. S. intermedius exhibits a reaction similar to S. aureus. Additional methods are required for differentiation.

Antibiotic Sensitivity Testing Procedure

There are two methods for performing antibiotic susceptibility testing. The direct inoculation of the Mueller Hinton plate with the clinical specimen can provide valuable preliminary susceptibility information when dealing with urgent clinical situations. The direct testing method may be used with any body fluid that is normally sterile such as urine. The indirect method utilizes standardized dilutions of specimens and is the preferred method for performing antibiotic sensitivity testing. This method yields results that correspond more closely to serum levels of the antimicrobials being tested.

1. Remove the Mueller Hinton plate from refrigerator and warm to room temperature to avoid moisture buildup after inoculation. Plates should be kept inverted (media on top) during storage and warming.
2. Carefully decant any moisture that accumulates in the plate lid during warming to prevent dripping on to the surface of the media.
3. Insure that antibiotic discs have been stored properly; i.e. refrigerated or frozen, and are within their expiration dates.
4. Dip a sterile swab into the urine (direct method) or standardized inoculum (Indirect method). **See notes in the following section regarding preparation of standardized inoculum.
5. Express excess fluid from the swab by rotating and pressing the swab firmly against the specimen container.
6. Streak the swab over the entire surface of the plate 3 times rotating the plate ¼ turn after each streak to obtain uniform coverage of the plate.
7. Allow the plate to dry for 3-5 minutes but no longer than 15 minutes before applying the sensitivity discs.
8. Using a single or multi-disc dispenser, drop 6 antibiotic discs onto the plate. If using a single disc dispenser, distribute evenly around the plate. Do not place more than 8 antibiotic discs on one plate.
9. To prevent discs from falling off of the plate when inverted, gently press discs into the media using a sterile swab.
10. Incubate inoculated plate at 37° C for 18-24 hours.
11. Following incubation, measure the diameter of the zone of complete inhibition (clear zone) to the nearest millimeter with a ruler or caliper. It is easier to measure from the back of the plate illuminated by a reflected light source.
12. Compare measured results to a database of established values for susceptibility and resistance. **Note: Do not assume that the largest zone of inhibition is indicative of the best antibiotic choice.*
13. Treat all inoculated plates as biohazardous material and dispose of properly.

Preparation of Standardized Inoculum (Indirect Sensitivity)

This is the preferred method for antibiotic sensitivity testing as it provides the most reliable results. The BBL Prompt® system (included) greatly facilitates preparation of a standardized inoculum. Please review all steps and separate detailed instructions for BBL Prompt® prior to use.

1. Remove one Prompt inoculation tube from the pack and place upright in a rack.
2. Remove one inoculation wand from the package being careful not to contaminate the tip.
3. Holding the wand tip perpendicular to the surface of the agar, gently touch five isolated colonies greater than 1 mm in diameter. This will fill the indentations on the tip of the wand with a precise number of bacteria.