

Technical Information

MUELLER HINTON AGAR W/ AND W/O BLOOD TI NO. 1620

9592

USE: Mueller Hinton Agar is useful in the cultivation of *Neisseria* species and sulfonamide testing as well as a basal media for susceptibility testing by the Kirby-Bauer method.

HISTORY: Mueller Hinton Agar was developed in 1941 primarily for isolation of pathogenic *Neisseria*. Since that time Mueller Hinton Agar's use in this capacity has diminished and the medium has been adopted for use in standardized disk anti-microbial agent susceptibility testing; therefore this medium with or without 5% sheep blood is recommended for susceptibility testing. The medium with added blood is recommended for more fastidious organisms.

PRINCIPLE: Mueller Hinton medium contains dried infusion of beef, acid hydrolysate of casein & soluble starch. Agar is a solidifying agent. The starch present acts as a "protective colloid" against toxic materials present in the medium.

FORMULA:

Beef-infusion from.....	300.0 g	Agar.....	17.0 g
Acid digest of casein.....	17.5 g	Starch.....	1.5 g
Sheep blood....(optional).....	50.0ml	Deionized water.....	1000ml

pH 7.3 ± 0.1 @ 25°C

PRECAUTIONS: This product is "For In Vitro Diagnostic Use" and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers and media after their use. Directions should be read and followed carefully.

STORAGE: This product is ready for use and no further preparation is necessary. The product should be stored in its original container at 2-8°C until used. Do not freeze or overheat. Allow product to come to room temperature before use. Do not incubate prior to use.

PRODUCT DETERIORATION: This product should not be used if (a) there is evidence of dehydration, (b) the product is contaminated, (c) the color has changed, (d) the expiration date has passed, or (e) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE AND TRANSPORTATION: The laboratory should provide suitable collection containers and instructions for their proper use. Specimens should be transported to the laboratory without delay and protected from excessive heat or cold— If there is any delay in processing a clinical specimen, the specimen should be placed in a suitable transport medium such as Amies, Stuarts, Cary-Blair, a pre-reduced media or gassed-out tube, Transgrow or Jembec System.

General rules applicable to all clinical specimens:

1. Quantity of specimen should be sufficient to permit thorough examination.
2. The specimen should be collected properly and should be representative of the infected area.
3. Care should be taken to prevent contamination of specimen.
4. Specimens should be taken to the laboratory promptly.
5. Specimens should be obtained before any antibiotics are administered to the patient. If therapy was initiated prior to collection, this should be noted on the forms sent with the specimen.

For further directions on the collection and transportation of specimens, consult the chapter "Collection, Handling and Processing of Specimens" in the ASM Manual, 3rd edition, 1980.

PROCEDURE: The usual clinical microbiological equipment is required for procedures involving this product. The media and equipment required will depend on the identification scheme employed by the microbiologist.

INSTRUCTIONS: Kirby-Bauer Method

1. Select at least four to five well-isolated colonies of the same morphological type from an agar plate culture. Touch the top of each colony with a wire loop and transfer the growth to a tube containing 4 to 5ml of a suitable broth medium, such as soybean-casein digest broth.
2. Allow the broth culture to incubate at 35 to 37°C until it achieves or exceeds the turbidity of a 0.5 McFarland Standard.
3. Adjust the turbidity of the actively growing broth culture with sterile saline or broth to obtain a turbidity visually comparable to that of the turbidity standard. To perform this step properly, use adequate light, and, to aid in the visual comparison, read the tube against a white background with contrasting black lines.
4. Within 15 min. after adjusting the turbidity of the inoculum suspension, dip a sterile nontoxic swab on an applicator into the adjusted suspension. Rotate the swab several times pressing firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.
5. Inoculate the dried surface of a Mueller-Hinton agar plate by streaking the swab over the entire sterile agar surface. Repeat this streaking procedure two more times, rotating the plate approximately 60 degrees each time to ensure an even distribution of inoculum. If the plate is satisfactorily streaked, and the inoculum was correct, the zones of inhibition will be uniformly circular and there will be a confluent or an almost confluent lawn of growth. If only isolated colonies grow, the inoculum was too light and you should repeat the test. Replace the plate top and allow 3 to 5 min., but no longer than 15 min., for any excess surface moisture to be absorbed.

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before applying the drug-impregnated disks.

NOTE: Avoid extremes in inoculum density. Never use undiluted overnight broth cultures for streaking plates.

6. Place the appropriate drug-impregnated disks on the surface of the agar plate. With sterile forceps or needle tip, gently press down each disk to ensure complete contact with the agar surface. You may place the disks individually or with a dispensing apparatus, but you must distribute them evenly so that they are no closer than 24mm from center to center. Because some of the drug diffuses almost instantaneously, do not move a disk once it has come in contact with the agar surface.

7. Invert the plates and place them in a 35C incubator within 15 min. after the disks are applied. The plates should not be incubated under an increased concentration of CO₂, because the interpretive standards were developed by using aerobic incubation and, with some agents, CO₂ will significantly alter the size of the inhibitory zones.

8. After 16 to 18 hours of incubation, examine each plate and measure the diameters of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Measure the zones to the nearest whole millimeter, using sliding calipers, a ruler, or a template prepared for this purpose, held on the back of the petri plate illuminated with reflected light against a black nonreflecting background. If the test organism is a *Staphylococcus* sp., use transmitted light to examine for light growth of methicillin-resistant strains. If blood is added to the agar base, measure the zones from the surface illuminated with reflected light, with the cover removed.

MATERIALS REQUIRED BUT NOT SUPPLIED: (1) Loop sterilization device, (2) Inoculating loop, swab, collection containers, (3) Incubators, anaerobic jars or candle jars, (4) Supplemental media, (5) Quality control organisms, (6) Susceptibility disks.

USER QUALITY CONTROL: Use "Instruction" procedures to inoculate media with *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853.

RESULTS: Interpret susceptibility according to standardized tables.

LIMITATIONS: When using the disk diffusion method, technical errors can compromise accuracy & reliability. The following are common sources of error encountered in clinical microbiology laboratories:

1. Improper storage of disks.
2. Inoculum adjusted too heavy or light.
3. Incubation deviating from 35C or use of an increased CO₂ atmosphere.
4. Premature reading of test results before the full 16-18 hours.
5. Clerical error in transcribing the control data.
6. Reader error when measuring zone diameters.
7. Deterioration of the turbidity standard.
8. Contamination or other changes in the control strain.

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CATALOG NUMBER(S):

01-620	Mueller Hinton	100x15mm Plate	(10/pkg)
01-622	Mueller Hinton w/Blood	100x15mm Plate	(10/pkg)
04-050	Mueller Hinton	150x15mm Plate	(10/pkg)
04-055	Mueller Hinton w/Blood	150x15mm Plate	(10/pkg)
06-1478	Mueller Hinton Broth-4ml	15x103mm Tube	(72/cs)
06-1480	Mueller Hinton Broth-4ml	15x103mm Tube	(12/pk)
08-594	Mueller Hinton Broth-10ml	20x113mm Tube	(72/cs)
08-596	Mueller Hinton Broth-10ml	20x113mm Tube	(12/pk)
09-592	Mueller Hinton Agar -25ml DEEP	20x150mm Tube	(12/pk)

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