Mueller Hinton II Agar

I INTRODUCTION

Mueller Hinton II Agar is used in the standardized disc diffusion procedure for determining the susceptibility of rapidly-growing aerobic organisms to antimicrobial agents.

PRODUCT INFORMATION

II INTENDED USE

Mueller Hinton Agar is recommended for antimicrobial disc diffusion susceptibility testing of common, rapidly growing bacteria.

NOTE: The recommended medium for disc diffusion susceptibility testing of *Streptococcus pneumoniae* is Mueller Hinton Agar with 5% Sheep Blood.

III SUMMARY AND EXPLANATION

Mueller Hinton Agar was originally developed because clinical microbiology laboratories in the early 1960s were using a wide variety of procedures for determining the susceptibility of bacteria to antibiotic, and chemotherapeutic agents, Bauer, Kirby and others developed a standardized procedure in which Mueller Hinton Agar was selected as the test medium. A subsequent international collaborative study confirmed the value of Mueller Hinton Agar for this purpose because of the relatively good reproducibility of the medium, the simplicity of its formula, and the wealth of experimental data that had been accumulated, using this medium.

The NCCLS has written a performance standard for the Bauer-Kirby procedure and this document should be consulted for additional details. The procedure is recommended for testing rapidly growing aerobic or facultatively anaerobic bacterial pathogens, such as staphylococci, members of the *Enterobacteriaceae*, aerobic gram-negative rods; e.g., and *Pseudomonas* spp.

IV PROCEDURE

Instructions

1. Perform a Gram stain before starting a susceptibility test to confirm culture purity and to determine appropriate test battery.
2. Examine plates after 16 to 18 h incubation. A full 24 h incubation is recommended for *Staphylococcus aureus* with oxacillin to detect methicillin-resistant *S. aureus* (MRSA) and for *Enterococcus* spp.
3. A confluent “lawn” of growth should be obtained. If only isolated colonies grow, the inoculum was too light and the test should be repeated. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disc, to the nearest whole millimeter, using sliding calipers, a ruler, or a template prepared for this purpose. The

measuring device is held on the back of the inverted plate over a black, non-reflecting background, and illuminated from above.

The endpoint should be taken as the area showing no obvious visible growth that can be detected with the unaided eye. Disregard faint growth of tiny colonies which can be detected with difficulty near the edge of the obvious zone of inhibition. *Staphylococcus aureus* when tested with oxacillin discs is an exception, as are enterococci when tested with vancomycin. In these cases, transmitted light should be used to detect a haze of growth around the disc which is shown by "occult resistant" MRSA strains or vancomycin-resistant enterococci. With *Proteus* species, if the zone of inhibition is distinct enough to measure, disregard any swarming inside the zone. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.