

**TITLE:** COMPARATIVE EVALUATION OF MH-SHEEP BLOOD AND MH-HORSE BLOOD (MH-F) FOR THE DISK DIFFUSION SUSCEPTIBILITY TESTING OF *STREPTOCOCCI* SPECIES

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**ABSTRACT:**

Antimicrobial susceptibility testing for fastidious microorganisms, such as *Streptococcus* spp., require supplemented medium to ensure bacterial growth. Mueller-Hinton agar (MH) plus 5% defibrinated horse blood and 20 mg/L  $\beta$ -NAD (MH-F) is recommended by The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST) for disk diffusion test of *Streptococcus pneumoniae*, *Streptococcus* groups A, B, C, G, and viridians group *streptococci*. In Brazil it is logistically cumbersome and expensive to obtain Muller-Hinton agar supplemented with horse blood. Alternatively, the Clinical and Laboratory Standards Institute (CLSI) recommends MH agar supplemented with 5% defibrinated sheep blood (MH-SC) as the standard medium for disk-diffusion test of *Streptococcus* spp. MH-SC has been used for many years and therefore is ready to obtain in Brazil. The aim of the present study was to validate the MH-SC medium as an alternative for antimicrobial susceptibility of *Streptococci*, in comparison to MH-F using the disk diffusion technique. The disk diffusion was performed in three triplicate experiments of each medium. Initially, a set of ATCC isolates (*S. pneumoniae* ATCC 49619, *S. agalactiae* ATCC 12386, *S. mitis* ATCC 6249, and *S. pyogenes* ATCC 19165) was tested in MH-SC and MH-F (PlastLabor). EUCAST/BrCAST recommendations were used for inoculum preparation, inoculation, incubation and reading. Zone diameters were read by two blinded operators. The following antibiotics (Oxoid) were tested: erythromycin (15  $\mu$ g), levofloxacin (5  $\mu$ g), vancomycin (5  $\mu$ g), ceftriaxone (30  $\mu$ g) – only for *S. pneumoniae*, and oxacillin (1  $\mu$ g) – only for *S. pneumoniae*. The *S. pneumoniae* was used as a quality control for validation of the experiments according to EUCAST/BrCAST. The results of the other *Streptococci* were used to compare the MH-SC and MH-F. The correlation between disk diffusion zone diameter between MH-F and MH-SC presented full agreement (1 to 2 mm of difference). These preliminary results indicate that MH-SC media present comparable results to MH-F for susceptibility testing of *S. pneumoniae*, *S. agalactiae*, *S. mitis*, and *S. pyogenes* using the EUCAST/BrCAST criteria. Clinical isolates will be evaluated to assess whether MH-SC also presents full correlation with MH-F.

**Keywords:** Fastidious Microorganisms; Horse Blood Agar; Medium Validation; Susceptibility Testing; Sheep Blood Agar

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