EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

Warning against the use of gradient tests for benzylpenicillin MIC in *Streptococcus pneumoniae*. Updated 20 August, 2025

EUCAST benzylpenicillin breakpoints in *Streptococcus pneumoniae* are S≤0.06 mg/L, R>1 mg/L for indications other than meningitis. Isolates which are positive in the screen for beta-lactam resistance (with the oxacillin 1 µg disk) have benzylpenicillin MIC values above 0.06 mg/L and are categorized either "susceptible, increased exposure", in which case isolates can be treated with benzylpenicillin if dosing is adjusted according to the MIC value, or resistant (R>1 mg/L), in which case benzylpenicillin, and often many other beta-lactam agents, should be avoided for treatment.

Until 2024, the EUCAST recommendation was to perform MIC determination for benzylpenicillin on all screen positive isolates. In 2024, EUCAST published zone diameter breakpoints for benzylpenicillin 1 unit to be used in screen positive isolates to categorise benzylpenicillin as "susceptible increased exposure" or "resistant". For laboratories still using MIC determination for benzylpenicillin SIR categorisation in *S. pneumoniae*, it is important to perform this with validated and accepted methods. This is especially important in isolates for which benzylpenicillin MICs are in the range 0.5 – 4 mg/L.

Following questions from NEQAS, EARS-Net and EUCAST users, the EDL investigated the accuracy of benzylpenicillin gradient tests Etest™ (bioMérieux) and MTS™ (Liofilchem). Both were tested on in-house prepared MH-F agar from Oxoid (Thermo Fisher Scientific) och BBL (BD). Broth microdilution using Mueller-Hinton-F (MH-F) broth was used as the reference.

Both gradient tests were found to frequently underestimate MIC values by one or more doubling dilutions. In the area around the R breakpoint (0.5 – 4 mg/L), and with some variation between the MH-F media and the two tests, 0 – 37% of values were on the reference MIC, 63 – 100 % were below and 0-10 % of the values above the reference MIC. Thus a clear negative bias in the test results. This is especially detrimental in the area close to the R breakpoint. Laboratories using gradient tests must be aware of this and MIC values of 0.5 - 4 mg/L should be checked with broth microdilution.

Disk diffusion: The oxacillin 1 µg disk screening test can be used to (1) screen for beta-lactam resistance and (2) to exclude resistance to several beta-lactam agents.

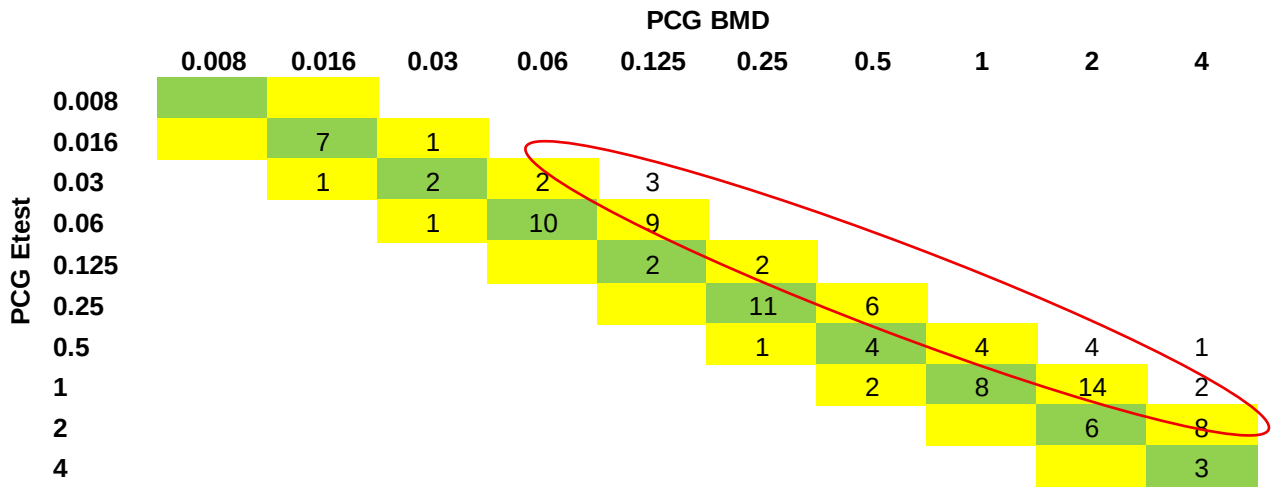
(1) ***S. pneumoniae* with an oxacillin 1 µg inhibition zone <20 mm** have beta-lactam resistance mechanisms. In these isolates, disk diffusion with benzylpenicillin can be used to categorise benzylpenicillin as "susceptible increased exposure" or "resistant". EUCAST advices against the use of gradient tests to categorise benzylpenicillin in screen positive isolates.

(2) ***S. pneumoniae* with an oxacillin 1 µg inhibition zone ≥9 mm** are not resistant to either of ampicillin, amoxicillin and amoxicillin-clavulanic acid, piperacillin and piperacillin-tazobactam, cefotaxime, ceftriaxone, cefepime, ceftaroline or ceftobiprole. These can without further testing, be reported "susceptible". However, isolates with zone diameters <9 mm are not automatically resistant to these agents and need further investigation.

Investigating the performance of gradient tests (Etest from bioMerieux; MTS from Liofilchem) on two different media vs. broth microdilution for the determination of benzylpenicillin MICs.

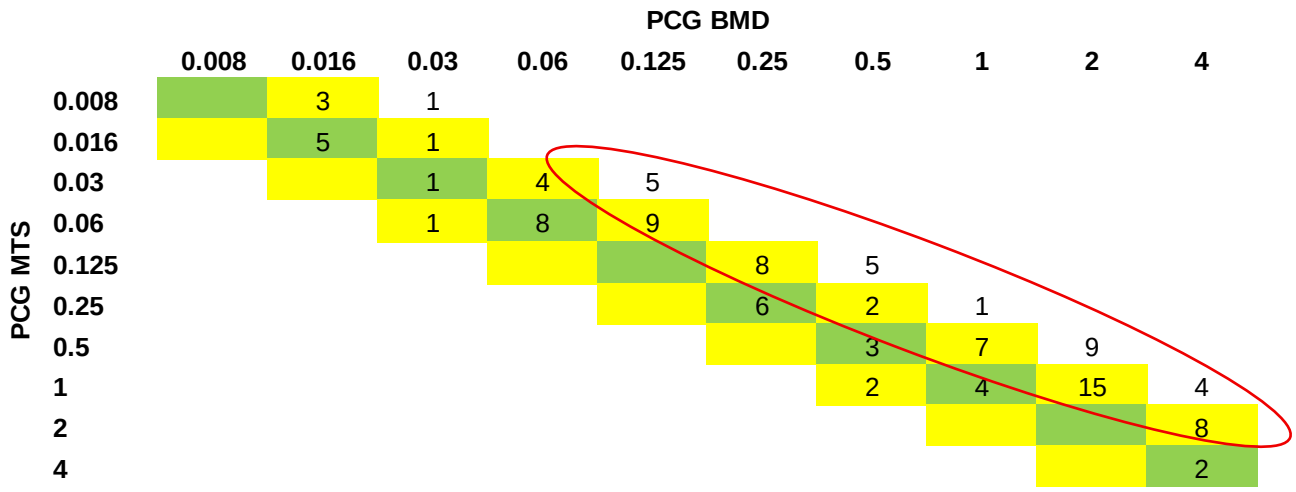
Essential agreement (EA) and bias were calculated according to ISO 20776-2 (2021). Acceptable limits are ≥90% EA and less than ±30% bias.

Etest, bioMerieux



Essential agreement	91%
Bias	-45%

MTS, Liofilchem



Essential agreement	78%
Bias	-69%