

Determination of urea concentration in BALF by colorimetric method

To determine the urea concentration, a commercially available kit (DIUR-500, BioAssay Systems) based colorimetric method was used in 96-well plates. This kit determines urea directly in biological samples without any pretreatment. For BALF, 50 µL water (blank), 50 µL 5 mg urea/dL (the 50 mg/dL standard diluted in water) and 50 µL samples were deposited in duplicate into separate wells. Then, 200 µL working reagent was added and mixed gently before incubating for 50 min at room temperature. The optical density (OD) at 450nm was then read using a LT-5000MS plate reader (Labtech, France). Urea concentration (mg/dL) of the sample is calculated as: $[Urea] = [(OD_{sample} - OD_{blank}) / (OD_{standard} - OD_{blank})] \times n \times [STD]$ (mg/dL). OD_{sample} , OD_{blank} and $OD_{standard}$ are OD values of sample, standard and water, respectively. n is the dilution factor. $[STD] = 5$ urea standard concentration (mg/dL). The dilution factor is calculated as: $dilution\ factor = [concentration\ of\ urea\ in\ plasma\ (mg/dL)] / [concentration\ of\ urea\ in\ BALF\ (mg/dL)]$. The concentration of urea in plasma we used was 50 mg/dL [1].

1. S.W.S. Yapa, J. Li, C.J.H. Porter, R.L. Nation, K. Patel, M.P. McIntosh, Population Pharmacokinetics of Colistin Methanesulfonate in Rats: Achieving Sustained Lung Concentrations of Colistin for Targeting Respiratory Infections, *Antimicrob Agents Ch*, 57 (2013) 5087-5095.