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Measurement of Evans blue dye concentration in central and peripheral tissue following administration in vitro and in vivo: dose- and replicate-dependent effects on absorbance, fluorescence, and extraction by trichloroacetic acid

Author Block: Hwai-Lee Wang[1], Ted Weita Lai[1,2]

[1]Graduate Institute of Clinical Medical Science, China Medical University, Taichung, Taiwan,[2]Translational Medicine Research Center, China Medical University Hospital, Taichung, Taiwan

Abstract:
The Evans blue dye (EBD) is the most commonly used inert tracer for tracking endogenous plasma protein extravasation into the brain. Even though several methods have been developed for measuring EBD concentration extravasated into central and peripheral tissues, the accuracy and precision of these methods remains poorly defined. First, we measured the absorbance and fluorescence of EBD solutions prepared in 96-well plates using standard photo-spectrometer. EBD absorbance increased in a dose-dependent manner, and logarithm-transformation revealed a sigmoidal curve with Hill coefficient of 2.086. The steepest slope of the curve lied between 5 µg/ml to 500 µg/ml, and hence this concentration range was the region at which measurements would be most precise. On the other hand, EBD fluorescence also increased in a concentration-dependent manner, but with region of precision from 0.2 µg/ml to at least 5 µg/ml. Nevertheless, substantial photo-bleaching of EBD fluorescence was found with increasing concentrations. Thus, our results recommends the use of fluorescent measurements to define EBD concentrations between 0.2 µg/ml to 5 µg/ml, and the use of absorbance measurements to define EBD concentrations between 5µg/ml to 500 µg/ml. Second, we determined the efficiency at which EBD can be retrieved from plasma proteins using the trichloroacetic acid (TCA) extraction technique. The degree of extraction was substantial even with 1:1 plasma-to-TCA ratio, and reached maximum with 1:2 ratio where increased ratios failed to further increase EBD extraction. Notably, contrary to earlier reports, we failed to completely extract EBD from plasma sample even with substantial amount of TCA. Third, we determined the sample-to-TCA ratio required to maximally extract EBD from central and peripheral tissue in rats receiving intravenous EBD. Taken together, the data presented here will be of great value in guiding future research using EBD as a tool to study central and peripheral vascular permeability.
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*** Sponsor First Name:** : Ted Weita

*** Sponsor Last Name:** : Lai

*** Sponsor Phone Number:** : +886 978-021-982

*** Sponsor Email:** : ted.weita@me.com
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