

# A protein dye-binding assay on cellulose membranes for tear protein quantification: Use of conventional Schirmer strips

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**PURPOSE:** To develop a method to quantify tear protein concentration with the sensitivity to measure this variable in the restricted volumes of single human tear samples. **METHODS:** Aliquots of tear fluid from healthy subjects and a solution of standard bovine serum albumin (BSA) were spotted on cellulose membranes. Membranes were fixed, stained for protein with Coomassie blue, and washed until they displayed clear backgrounds. Stained spots were excised and eluted in a defined volume of methanol-ammonia, and the absorbance was determined spectrophotometrically at 610 nm. Membranes were calibrated by calculating their apparent thickness from the areas of stained spots and the corresponding aliquot volumes of either tear fluid or BSA solution. **RESULTS:** In our dye-binding assay, absorbance (0-1.00 OD) was found to have a linear relation with tear fluid volume (1-7  $\mu$ L). In a study involving samples from 33 healthy subjects, aliquots (3  $\mu$ L) of tear fluid were found to yield absorbances in the