Comparison of low-abundance biomarker levels in capillary-collected nonstimulated tears and washout tears of aqueous-deficient and normal patients.

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**Purpose:** Low tear volume limits the use of nonstimulated (NS) microcapillary tear collection in aqueous-deficient (AD) patients. Adding a small amount of "washout" fluid to the eye prior to tear collection is a potentially viable alternative method for abundant proteins, but is relatively untested for low-abundance biomarkers. This study determined the feasibility of the washout (WO) method as an NS alternative for low-abundance biomarkers. NS and WO biomarker profiles were compared between AD patients and non-AD controls to determine if the two methods identify the same intergroup differences.

**Methods:** Matching NS and WO tears were collected from 48 patients by micropipette, the WO sample after instillation of 10 μL saline. Tear cytokine levels were measured by 27-Plex Bio-Rad assay. Bland-Altman analyses for each biomarker determined the agreement between tear sample types. Patients were grouped as AD or non-AD based on Schirmer score to determine if NS profile between-group differences were preserved in WO tears.

**Results:** Bland-Altman plots showed good biomarker level agreement between NS and WO tears for most cytokines. Five biomarkers, among those most often cited as differing in AD dry eye, differed significantly between non-AD and AD...
groups in both tear types. Additional biomarker differences were seen in NS tears only.

**CONCLUSIONS:** The WO tear collection method is a viable alternative to NS tears for many low-abundance biomarkers and is able to replicate major NS tear differences between dry eye groups. More subtle intergroup differences are lost in WO samples because of reduced statistical power.

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