# Method Optimization for the Quantification of Total Protein Deposited on Silicone Hydrogel Contact Lens Materials


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## Introduction

- Silicone-hydrogel (SH) contact lens materials such as balafilcon (Purevision (PV), Bausch & Lomb) and lotrafilcon (Focus Night&Day (FND), CIBA Vision) deposit significantly less protein compared to conventional hydrogel contact lens materials such as etafilcon (Acuvue (AV), Vistakon).[1,2] Preliminary data from our laboratory suggests that PV and FND lenses deposit less than 30µg and 5µg of total protein respectively, which is in stark contrast to AV lenses, that tend to deposit over a 1000 µg of total protein.
- In order to gain a thorough understanding of protein deposition on SH lenses, it is advantageous to assess multiple variables simultaneously. Thus the amount of individual or true protein actually available for assay is only a fraction of the total amount deposited, as the extracted deposit must be "divided" into various fractions to facilitate multiple assays.
- Traditional protein assays, such as the Bradford[3] and micro-BCA[4], lack the required sensitivity to quantify the nanogram quantities of total protein expected in a fraction of a SH lens extract.
- Factors important to the measurement of protein in contact lens extracts include the effect of tear film components such as lipid[1,5] and mucin[6] as well as cross-reactivity to SH polymer components co-extracted with protein in extracts of unworn SH contact lenses.

## Aims

- To optimize a procedure to accurately quantify total protein in extracts from SH lenses in the low to mid nanogram range.

## Methods

- Four protein assays were assessed: (a) CBQCA (Molecular Probes); (b) red-dot-blot[7] (RDB); amido black (AB) onto (c) nitrocellulose (NC) and (d) polyvinylidene difluoride (PVDF) membranes.
- The CBQCA assay was performed according to the manufacturer's instructions in 96-well ELISA microtitre plates. The ELISA plates were read on an Alpha Innotech Fluochem 8000 (abs/em 465/550 nm).
- For membrane-based assays (c) and (d), protein was applied to membranes in phosphate-buffered saline (PBS; 50 µl) using the Bio-Dot Microfiltration Apparatus (BIO-RAD). For the RDB assay, protein was applied manually to nitrocellulose in 1 µl of PBS. Membranes were imaged and densitometry was performed on a Syngene Gene Genius™ Gel Documentation System.
- Assays were evaluated for sensitivity by titration of BSA standard. The working range (25 – 500 ng) was investigated for select assays with BSA, hen egg lysozyme (HEL) and bovine lactoferrin (all data not shown).
- To evaluate accuracy, protein assays were subjected to a panel of tear film proteins analyzed at 200 or 300 ng. These proteins included human lysozyme, human secretory IgA, human albumin, human tears collected from healthy volunteers, and mucin from bovine submaxillary glands. Protein solutions were prepared in saline at ~1 mg/ml and quantified by Ninhydrin[8] assay using BSA as standard.
- Cross-reactivity toward 50:50 acetonitrile / 0.2% trifluoroacetic acid[9] extracts (1.5 ml) of unworn PV, FND and AV contact lenses was assessed by measuring apparent protein.
- The tolerance of protein assays to the presence of lipid was tested by measuring HEL in the presence and absence of lipid at final concentrations consisting of triolein (0.032 mg/ml), cholesterol (0.112 mg/ml), oleic acid (0.016 mg/ml), oleic acid methyl ester (0.048 mg/ml), and cholesteryl oleate (0.15 mg/ml). Select assays were compared for their ability to measure proteins with complex characteristics such as human α1-acid glycoprotein (pI of 2.7 and 50% carbohydrate).

## Results

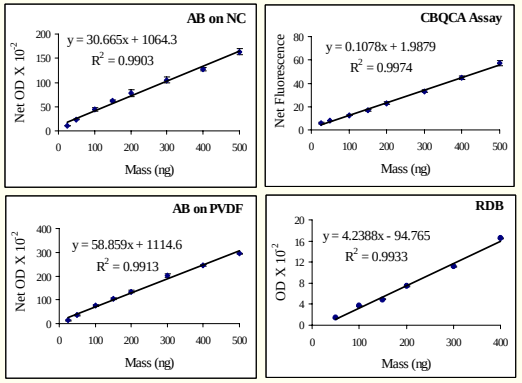


**Figure 1: Comparison of Assay Sensitivity:** Graphical representation of the lower limits of detection using the protein standard BSA for the four protein assays CBQCA, RDB, AB on PVDF and AB on NC under study.

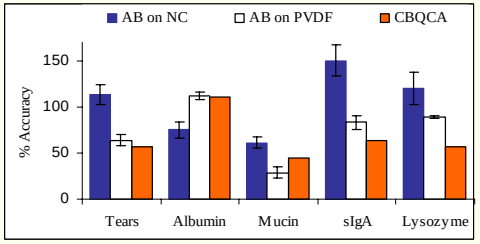


**Figure 2: Evaluation of Protein Assay Quantitative Accuracy:** A fixed mass of protein was subjected to protein assays AB on NC, AB on PVDF, and CBQCA. BSA served as standard. Performance was based on the ability of each assay to measure protein concentration relative to the Ninhydrin assay. Percent accuracy = [Measured Mass / Applied Mass] X 100. The average % accuracy and standard deviation are given for AB on NC and AB on PVDF (n=3); for CBQCA, n=1.

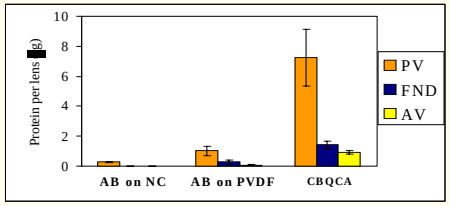


**Figure 3: Cross-Reactivity of Protein Extracts from Unworn Contact Lenses:** Graphical representation of apparent protein measured by assays AB on NC, AB on PVDF and CBQCA from extracted unworn lenses (PV,FND,AV). The amount of lens extract assayed was 1.6-10% for PV and AV and 8-16% for FND. Each value represents the average and standard error of the mean (SEM) (n=4).

| | % Accuracy of Measurement | | |
|---|---|---|---|
| | AB on NC | AB on PVDF | CBQCA |
| HEL | 117 ± 8 | 100 ± 6 | 98 |
| HEL + Lipid | 125 ± 14 | 95 ± 7 | 102 |
| acid glycoprotein | 12 ± 3 | 76 ± 3 | 65 |
| n | 3 | 2 | 1 |

**Table 1: Evaluation of Lipid and Complex Protein Structure on Protein Assay Quantitation:** Protein was applied and analyzed as in Figure 2. The lipid mixture is described in the Methods.

## Conclusions

- All protein assays were sensitive in the low nanogram range. The RDB could not be used with the Bio-Dot Microfiltration apparatus and was discontinued part way through this study.
- AB on NC (and RDB) most accurately measured the mass of human tears and mucin.
- AB on PVDF showed the least protein-to-protein variation and, in general, measured proteins, including α1-acid glycoprotein, more accurately than CBQCA.
- All protein assays measured protein in the context of lipid at two times the concentrations typically present in human tears.
- Cross-reactivity to extracts was observed with all assays except AB on NC.
- We conclude that the optimized AB on NC assay provides optimal reliability and sensitivity for quantification of a minimum of 50 ng total protein. This assay will be very useful to quantify total protein deposition on SH lens materials.

## References


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