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Protein – analysis in Aqueous Humor (AH)

The analysis of aqueous humor has a long tradition. The well known Goldmann- Witmer Index for detection of intraocular antibody synthesis is known since more then 50 years . With modern techniques for analysis and interpretation of AH data it is possible to support diagnosis of inflammatory eye diseases, and improve research on eye diseases as recently shown by successful discovery of the cause of Fuchs heterochromic Cyclitis.
The small volume of AH available from the single patient demands for a very sophisticated analytical approach, restricted to the specialized laboratory.
The following procedures represent the experience of the Neurochemistry Laboratory in Göttingen (Prof.H. Reiber) and the Ophthalmologist Dr. C.Quentin (University hospital Göttingen).
Ref. CD Quentin and H.Reiber, Fuchs heterochromic cyclitis: Rubella Virus Antibodies and Genome in Aqueous Humor. Am. J. Ophthalmology 2004; 138: 46-54

ANALYSIS
Usual **sample volumes**: 50-200 µl
This small volume makes it necessary to modify the relevant assays from CSF analysis and restrict the wishful analysis to the necessary minimum.

Cell count in AH: is less important then in CSF analysis as the Ophthalmologist has a chance to see increased cell count by direct inspection of the eye.
Total protein analysis consumes only a small volume and helps for overview and planning of further analysis. : 5µl AH + 45µl NaCl predilution before analysis.
Basic Programm: Predilution to 150-200 mg/L total protein may help to save sample volume: so occasionally 150- 200 µl can be afforded for Nephelometric analysis of albumin and IgG (IgA and IgM are often not analysed to save volume for other tests).
Oligoclonal IgG is performed from undiluted AH (10µl) as performed for CSF with isoelectric focussing and immunoblot or silverstain.
Specific Antibody analysis in AH :
In the commercial ELISA 5 µl AH are directly applied into the well of a microtiter plate and 145 µl dilution buffer (particular buffer) are added. The residual procedures of an ELISA are not changed.
Specific Antibody analysis in AH is the most relevant assay in diagnosis of inflammations of the eye and usually needs albumin and IgG analysis for calculation of the Antibody Index (extended Goldmann-Witmer Index).If this is not possible (to small volume) the analysis of several antibody species are analysed in parallel as reference to detect intraocular synthesis, e.g. by an increased rubella -specific AH/Serum quotient more than 50% larger as other specific AH/Serum quotients like those from measles or VZV.

Evaluation of AH protein data in CSF Quotientdiagrams is possible to detect intrathecal IgG , IgA or IgM synthesis. A different reference range for the albumin quotient must be considered..