## Tear proteins other than lactoferrin - lipocalin

### TO DIAGNOSE
Test used to diagnose – e.g. Meibomian gland disease (MGD).

### VERSION of TEST
[V 1 ]

### DESCRIPTION
Tears are collected into microcapillary tubes and the concentration of lipocalin analysed after separation of proteins by high performance liquid chromatography. Lipocalin “fraction” determined by comparison to elution of standards such as lysozyme and lactoferrin.

### CONDUCT of TEST
1. Tears are drawn into the tube by capillary action from the inferior tear meniscus at the outer canthus.
2. Care should be taken to avoid stimulation of tears during this collection, and therefore it may be necessary for the subject to gain experience in the technique.
3. After collection, tears are usually centrifuged at 1,000 g for 5 min to remove debris, then aliquoted into smaller volumes and can be stored at –80°C until needed (No centrifugation step cited in the Yamada article, although it is used elsewhere).
4. HPLC conducted using TSK 3000SWx1 column (Tosoh Inc. Tokyo, Japan) and elution performed with 0.5M NaCl and 0.1M Na phosphate buffer (pH 5.0).

### Materials:
- Glass Microcapillary tubes - total volume <50ul
- HPLC
- UV spectrophotometer to measure absorbance at 230nm

### Variations of technique
It is also possible to collect tears using sponges or Schirmer strips.

### Standardization
<table>
<thead>
<tr>
<th>Time of day</th>
<th>Temperature</th>
<th>Humidity</th>
<th>Air speed</th>
<th>Illumination</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ X ]</td>
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</table>

### Diagnostic value
This version : [ ]
Other version: [ X ]

### Repeatability
Intra-observer agreement. [NA]
Inter-observer agreement. [NA]

### Sensitivity
[NA]

### Specificity
[NA]

### Other Stats
Mann Whitney U test – obstructive MGD and Seborrhoeic MGD have less lipocalin than normals. Amount of lipocalin correlated with tear film TBUT (r=-0.67; p=7.8x10^{-6}) and fluorescein scores (r=-0.74; p = 1.8x10^{-7}) Note: not correlated with Schirmer or cotton thread tests, or...
<table>
<thead>
<tr>
<th>Test problems</th>
<th>Control of tearing may be important although, as lipocalin is a regulated protein, the effect is minimised. HPLC fractions may contain other species.</th>
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</thead>
<tbody>
<tr>
<td>Test solutions</td>
<td>Tests that incorporate more than one measure of specific tear proteins may be more appropriate – e.g. lipocalin concentration together with lactoferrin concentration. Or a regulated versus constitutive (e.g. sIgA) protein test. Lipocalin concentration (as measured by ELISA) seems to increase in tears of contact lens wearers who are intolerant to lens wear. Lipocalin concentration (ELISA method) was more variable in menopausal women than in menopausal women who were on hormone-replacement-therapy. A thicker more stable lipid layer might be associated with lower levels of lipocalin in this group. Note: in this study, the authors found no significant differences between groups in the tear lipocalin concentrations or in the lipid layer appearance. Lipocalin 1 also appears to be secreted by the meibomian gland.</td>
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**References**


