### Tear protein (especially lactoferrin) analysis

**Test**
Test used to diagnose – e.g. aqueous tear deficiency (ATD).

**Version**  
[V 1 ]

**Description**
Tears are collected into microcapillary tubes and the concentration of lactoferrin (or other proteins) analysed by enzyme-linked immunosorbent assay.

**Conduct of test**
1. The subjects head is tilted to one side and a microcapillary tube (total volume of liquid that can be held <50ul) gently rested on the lower lid.
2. Tears are drawn into the tube by capillary action from the lower tear prism at the outer corner of the eye.
3. The time taken for tears to reach a specified point (3 - 5 µl) is recorded as flow rate (µl/min).
4. 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.
5. 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
6. Tears and lactoferrin standards are then processed by ELISA according to the instructions of the manufacturer of the kit (e.g. Oxis International Inc. Portland OR. USA)

**Materials:**
- Glass Microcapillary tubes - total volume <50ul
- Lactoferrin test kits from e.g. Oxis International Inc. Portland OR. USA.
- Microtitre plate reader – to measure absorbance at specific wavelengths (dependent on kit used)

**Variations of technique**
It is also possible to collect tears using sponges or Schirmer strips.  
It is also possible to design in-house ELISAs  
Lactoplate is a commercial technique

**Standardization**
- Time of day [ X ]  
- Temperature [ ]  
- Humidity [ ]  
- Air speed [ ]  
- Illumination [ ]  
- Other:[Rate of tear collection ]

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

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

**Sensitivity**
(true positives)  
[79.4% when using cut off value of 1.1mg/ml]  
Wang et al., 2005

**Specificity**
(100 – false positives)  
[78.3% when using cut off value of 1.1mg/ml]  
Wang et al., 2005

**Other Stats**
Significant decrease in lactoferrin concentration in Sjögren’s,  
Ohashi et al., 2003
<table>
<thead>
<tr>
<th>Test problems</th>
<th>Control of tearing is important – reflex tearing may influence results (although some proteins e.g. lactoferrin are regulated proteins which minimises this problem)</th>
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<tbody>
<tr>
<td>Test solutions</td>
<td><strong>FORWARD LOOK</strong> 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. The lactoferrin test is perhaps best used to discriminate between normals and severe dry-eye sufferers (e.g. Sjogrens etc). This test does not appear to be useful for mild dry-eye (as determined by contact lens wear intolerance) and normals</td>
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**References**

