DEWS	**DRY EYE: DIAGNOSTIC TEST TEMPLATE**	
RAPPORTEUR	Franz Grus	5th April 2006
TEST	**Tear Protein Profiles**	
TO DIAGNOSE	1. To identify objective changes in tear protein profiles in Contact Lens wearers and Dry Eye states. Of use in therapeutic trials. 2. Test used to distinguish between dry eye and non-dry eye subjects 3. Test used to distinguish between aqueous deficient and lipid deficient dry eye and non-dry eye subjects	REFERENCES
VERSION of TEST	[V 1]	Grus et al., 2006
DESCRIPTION	Tears are collected on Schirmer papers or by microcapillaries without touching the eye-lid (volume ≤ 5 µl). Tears are eluted from Schirmer papers and analyzed by surface-enhanced laser desorption/ionisation time-of-flight mass spectrometry (Seldi-TOF). For identification, the tear isolated by SDS-PAGE electrophoresis, eluted from the gel, reprofiled on Seldi-Protein Chips (to assure that the right biomarker was isolated), tryptic digested and identified by Maldi-TOF. Several hundreds of tear proteins and peptides can be detected and from these, a biomarker panel can be created by pattern-matching algorithms which can differentiate between the tears obtained in different clinical states. From several hundred detected proteins and peptides, a biomarker panel may then be constructed, using artificial neural networks, which can be used to discriminate between the different subtypes of dry-eye and healthy subjects and also to explore different conditions of contact lens wear. 1. Contact lens Wear and dry eye. In the study cited, (Grus et al 2005b) this biomarker panel was used to test the effect of using different contact lens cleaning solutions. Whereas in most of the solutions tested no significant effect could be observed, contact lens cleaning solutions containing moisturizers produced a highly significant effect on tear protein patterns. While at the start of the study, approximately 98% of tear samples were recognized as typical contact lens wearers, within a 4 weeks period, around 50% of participants in this group recovered to show a normal tear protein profile. These results were successfully validated in a completely independent study population. 2. Differentiating Dry Eyes from Normals Protein profiling shows differences between tears collected from dry eye and control subjects. Besides other known proteins such as lysozyme, proteins of the proline-rich protein families (PRP3 and 4) were found to be decreased in dry-eye patients accompanied by lower levels of nasopharyngeal carcinoma-associated proline-rich protein in tears of dry eye subjects. Higher levels of alpha-1-antitrypsin and calgranulin in tears of dry eye subjects.	

	3. Test used to distinguish between aqueous deficient and lipid deficient dry eye and non-dry eye subjects. The biomarkers used for this purpose, consist mainly of proteins and fragments of proteins from the proline-rich protein (PRP) family. In a recent study, a mutation or post-translational modification was found, which is more frequently found in the tears of dry-eye patients than in healthy subjects (Two amino-acids were exchanged in PRP4 fragments, Grus et al., 2005a).	
CONDUCT of TEST	i. After collection, tears are usually centrifuged at 1,000 g for 5 min to remove debris and can be stored at –80°C until needed. Schirmer papers can be frozen as they are. ii. Seldi-TOF is performed and biomarkers from the panel above identified. The artificial neural network, trained using well-characterised samples, provides a probability value indicating whether an 'unknown' pattern is consistent with a dry-eye state, contact lens wear or a normal eye.	
Web Video	Not available	
Materials:	• Schirmer strips • SELDI-TOF	
Variations of technique	It is also possible to collect tears using sponges or microcapillary tubes.	
Standardization	Time of day [X] Temperature [ ] Humidity [ ] Air speed [ ] Illumination [ ] Other:[Rate of tear collection]	
Diagnostic value	This version : [x] Other version: [ ]	
Repeatability	Intra-observer agreement. [NA] Inter-observer agreement. [NA]	
Sensitivity	[90]	
Specificity	[90]	
Other Stats	Multivariate discriminant analysis and artificial neural networks	
Test problems	Seldi-TOF Reader required	
Test solutions	This test may be able to differentiate between different dry eye states and also clinical conditions of contact lens wear. It may be possible to provide biomarkers which will allow the therapy of dry eye to be assessed in an objective manner.	
FORWARD LOOK	Because the biomarkers are already identified, low cost mass spectrometers can be used to do the analysis of tear samples. These will be available in the near future at affordable prices. Furthermore, it may be possible to integrate these markers in microfluidic analysis e.g. in test strips. Because a very high sensitivity is needed and only a limited sample volume is available, setting up an ELISA test for these biomarkers might not be very promising. As an alternative, it could be done by microarray analysis, but this might be more costly than using low-cost Mass Spectroscopy.	
Glossary		

References

Grus FH, Podust VN, Bruns K, Lackner K, Fu S, Dalmasso EA, Wirthlin A, Pfeiffer N. SELDI-TOF-MS ProteinChip Array Profiling of Tears from Patients with Dry Eye. *Invest Ophthalmol Vis Sci* 2005a;46:863-76.

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Grus et al. 2006, submitted for publication