DEWS	**DRY EYE: DIAGNOSTIC TEST TEMPLATE**	
RAPPORTEUR	Christophe Baudouin	7th Nov 2004
Reviewers	-	
TEST	**Flow cytometry in impression cytology**	
TO DIAGNOSE	Conjunctival inflammation / apoptosis	REFERENCES
VERSION of TEST	[V 1] [V2] Also available: Brush cytology for cell collection before flow cytometry procedures (Fujihara et al., 1997).	Baudouin et al. 1997 ; Fujihara et al., 1997
DESCRIPTION	This technique is highly sensitive and specific for analyzing expression of any marker by conjunctival epithelial cells, or identification of inflammatory and goblet cells. HLA DR normally not or weakly expressed. Strongly overexpressed in case of ocular surface inflammation	
NATURE of STUDY	Technique especially relevant in dry eye, allergy or assessment of antiglaucoma eyedrops	Brignole et al. 2000, 2001
CONDUCT of TEST	1. Without or under topical anaesthesia with one drop of 0.04% oxibuprocaine, one or more filters, 13 x 6.5 mm in size, are gently applied to the conjunctival surface. 2. After removal, the membranes are dipped into tubes containing 0.05% paraformaldehyde. The tubes have to be kept at 4°C before and after impression collection in order to avoid sample degradation during the phase of fixation. Under this condition the filters with the conjunctival specimens can be stored several days and sent to the laboratory in cold-conditioned containers before being processed for flow cytometry analyses. 3. Cell extraction is manually conducted by gentle agitation. After centrifugation in PBS, conjunctival cells are then immunostained and analyzed by flow cytometry. 4. Indirect or direct immunofluorescence procedures may be used. Simple or multi-color analysis can be performed commonly using 2 to 4 antibodies conjugated with different fluorochromes. A nonimmune isotype-matched mouse immunoglobulin has to be used as a negative isotypic control, fluorochrome-conjugated or not, according to direct or indirect immunofluorescence procedure. 5. At the end of incubation with specific antibodies, cells are centrifuged in PBS (1600 rpm, 5 minutes), resuspended in PBS and analysed on a flow cytometer. Intracytoplasmic markers can also be detected by using specific permeabilization techniques, such as 0.5% saponin, 100 triton X or ethanol. 6. Many markers available giving relevant information on ocular surface disorders; HLA DR expression by epithelial cells, gold standard for inflammatory assessment	Brignole et al. 2004
Web video	Not available	
Materials:	1. Polyethersulfone filters (Supor®, Gelman Sciences Ann Arbor, MI, USA), 13 mm in diameter with	

	pores of 0.20 µm 2. Paraformaldehyde freshly prepared and preserved at 4°C, monoclonal antibodies and material for immunostaining 3. Flow cytometer	
Standardization	Nil additional	
Variations of technique	[V2] Brush cytology for cell collection before flow cytometry procedures.	Fujihara et al., 1997
Diagnostic value	This version : [√] HLA DR inferior to 45% of positive cells and 18,000 MESF (molecular equivalent of soluble fluorochrome) in normal eyes. Widely above these values in inflammatory ocular surface disorders.	Brignole et al. 2004
Repeatability	Standardized technique reliable over time and from one laboratory to another	
Sensitivity	**(true positives)** [NA]	
Specificity	**(100 – false positives)** [NA]	
Other Stats	-	
Test problems	This procedure is highly technical and requires laboratory equipped with a flow cytometer and a staff familiar with immunostaining processing and flow cytometry analysis on paucicellular specimens	
FORWARD LOOK	Many markers for a large variety of applications have yet to be tested with further improvement of pathophysiological knowledge of ocular surface diseases	

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