Methodologies to Diagnose and Monitor Dry Eye Disease:
Report of the Diagnostic Methodology Subcommittee of the International Dry Eye WorkShop (2007)

ABSTRACT **The role of the Diagnostic Methodology Subcommittee of the Dry Eye Workshop was 1) to identify tests used to screen, diagnose and monitor dry eye disease, 2) to establish criteria for test performance, and 3) to consider the utility of tests in a variety of clinical settings. The committee created a database of tests used to diagnose and monitor dry eye, each compiled by an expert in the field (rapporteur) and presented within a standard template. Development of the templates involved an iterative process between the Chairman of the subcommittee, the rapporteurs, and, at times, an additional group of expert reviewers. This process is ongoing. Each rapporteur was instructed on how to the complete a template, using a proforma template and an example of a completed template. Rapporteurs used the literature and other available sources as the basis for constructing their assigned template. The Chairman of the subcommittee modified the template to produce a standardized version and reviewed it with the rapporteur. The completed database will be searchable by an alphabetical list of test names, as well as by functional groupings, for instance, tests of aqueous dynamics, lipid functions, etc. The templates can be accessed on the website of the Tear Film and Ocular Surface Society (www.tearfilm.org). This report provides a general overview of the criteria applied in the development of tests for screening and diagnosis.**

KEY WORDS **diagnosis, dry eye, Dry Eye WorkShop, methodology for appraising dry eye tests, questionnaires, tests for dry eye, screening, Sjogren syndrome**

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Diagnostic Methodology Subcommittee members: **Anthony J. Bron, FCOphth, FMedSci (Chair)**, Diagnostic Tests Section: **Anthony J. Bron, FCOphth (Captain)**; Mark B. Abelson, MD; George Ousler, BS; E. Pearce, PhD; Alan Tomlinson, PhD, DSc; Norihiko Yokoi, MD, PhD. Symptoms Section: **Janine A. Smith, MD (Captain)**; Carolyn Begley, OD; Barbara Caffery, OD; Kelly Nichols, PhD; MD; Debra Schaumberg, PhD; Oliver Schein, MD, MPH, MBA. Emerging Technologies Section: **Margarita Calonge, MD (Captain)**; Christophe Baudouin, MD, PhD; Eiki Goto, MD; Franz Grus, MD, PhD; Jerry Paugh, PhD, OD.

Writing Team Coordinator: Debra Schaumberg, PhD.

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Proprietary interests of Subcommittee members are disclosed on pages 202 and 204.

Reprints are not available. Articles can be accessed at: www.tearfilm.org.

Correspondence related to this chapter of the DEWS Report should be directed to: Anthony Bron, FMedSci, FRCS, Nuffield Laboratory of Ophthalmology, Walton St., Oxford OX2 6AW, UK. Email: anthony.bron@eye.ox.ac.uk

I. INTRODUCTION

The Diagnostic Methodology Subcommittee set out to create a detailed register of diagnostic tests used to diagnose and monitor dry eye. The aim was to perform a thorough review of the literature and other available sources, to summarize findings in a standardized fashion, and to provide the research community with a searchable database of tests, including an assessment of their diagnostic efficacy. The committee considered the feasibility and operational use of tests and questionnaires in a variety of settings, including general eye clinics, dry eye specialty clinics, clinical trials in dry eye, and non-trial clinical research in dry eye. The committee also sought to identify areas in which new tests are needed, and to provide advice on how these might be brought to clinical use.

The attempt to meet these goals has been challenged by the longstanding lack of a uniform set of criteria for the diagnosis of dry eye, for which there has been no generally agreed "gold standard." Studies of test efficacy and/or performance are influenced by the fact that subjects have often been selected based on the same tests that are under scrutiny. Similarly, the performance of any "new" test may be compromised when the test is assessed in a population of dry eye patients who have been diagnosed using unestablished criteria.

An additional challenge relates to the variety of settings in which diagnostic tests are being used. For example, tests may be applied in everyday clinical practice, or to assess eligibility in a clinical trial. Furthermore, tests may be used to follow the natural history of the disorder or to quantify clinical changes over the duration of a clinical trial (ie, in monitoring). Tests that are useful in one setting may differ from those employed in others.

OUTLINE

Table 1. Goals and objectives of the Diagnostic Subcommittee

To create a register of diagnostic tests used in dry eye diagnosis with the following characteristics:
- *A searchable register of referenced tests*
- *Variable sorting, eg,*
  - Alphabetical by test name
  - By organ system tested
    - Aqueous dynamics
    - Tear stability
    - Tear composition
    - Meibomian gland function, etc.
  - By utility, eg,
    - Diagnostic classification criteria
    - Clinical trials
      - Recruitment—entry criteria
      - Outcome measures
      - Monitoring specific drug actions, eg, anti-inflammatories; secretagogues
  - Natural history
- *Identification of evidence level*
  - [this will be a second phase of development]
  - —validation/precison and accuracy of tests
  - —system used

To consider the operational use of tests in different clinical environments
- *In general clinics*
  - What tests are feasible?
  - What questionnaires can be made available?
- *In dry eye clinics*
  - What tests are feasible?
  - What questionnaires can be made available?
- *In clinical trials*
  - Selection of tests
  - Order of tests
- *In non-trial Clinical Research*

Manuals of operation for individual tests
- Consider for selected, key tests
- Interface with industry

Future prospects
- What new tests are needed?
- How can they be brought to the general clinic?

II. GOALS OF THE DIAGNOSTIC METHODOLOGY SUBCOMMITTEE

The goals of the Diagnostic Methodology Subcommittee were to identify tests used to screen, diagnose, and monitor dry eye disease, and to establish criteria of test performance (test efficacy) and to consider their practical use in a clinical setting (Table 1).

To achieve these goals, the committee created a database of tests used in the diagnosis and monitoring of dry eye, each compiled by an expert in the field (rapporteur) and presented within a standard template. An alphabetical list of these tests can be found in Appendix 1, and Appendix 2 re-presents them in functional groupings, for instance, tests of aqueous dynamics, tests of lipid functions, etc.

III. DEVELOPMENT OF THE TEMPLATES

Templates were developed by an iterative process between the Chairman of the subcommittee and the rapporteurs. Each rapporteur was sent a set of instructions on how to complete a template, together a proforma template (Appendix 3) and an example of a completed template. Rapporteurs sent their completed templates to the Chairman of the subcommittee, who saved the original version and then modified it to correct any idiosyncrasies and produce a standardized version. A few tests have been covered by more than one rapporteur. The templates were then reformatted to remove redundant material or to add new sections, which are incorporated into the listing provided in Appendix 1. To facilitate searches, template files are titled by the test they describe. The table of functional groupings will enable investigators to identify a battery of tests that explores the influence of dry eye on a number of physiological indices (Appendix 2).

The full complement of templates can be accessed on the website of the Tear Film and Ocular Surface Society (www.tearfilm.org). It is expected that modifications will be made to these templates from time to time as new information becomes available.

Template headings (some of which are not currrently supplied with data) include the following:

1) The name of the original rapporteur;
2) The names of additional reviewers, where available;
3) The name of the test;
4) The purpose of the test;
5) The version of the test;
6) A short description of the test;
7) Details of studies conducted using the test, if relevant;
8) Details of the conduct of the test;
9) A statement of study results, if relevant;
10) A statement as to whether a web video is available, if relevant;
11) A list of the materials required for the performance of the test;
12) Variations of technique, if applicable;
13) Standardization—an indication of factors that could influence the test result, which, if standardized, could improve the efficacy of the test (eg, time of day, humidity, temperature, air flow, level of illumination, aspects of patient instruction, etc.).

The next sections relate to the performance of the test:

14) "Diagnostic value of the test" in practice, used, for instance, in conjunction with other tests;
15) Repeatability of the test;
16) Sensitivity of the test using a given cut-off value;
17) Specificity of the test using the same cut-off value (100—the false positive rate);
18) Other statistical information, if available.

Next, follows:

19) A box headed "Level of Evidence" for future use. Currently, this box is unused on all templates, since, at the time of writing, evidence criteria for the classification of tests, equivalent to those applicable to clinical trials, are not available.

The final section asked the rapporteur to identify:

20) Test problems encountered;
21) Any proposed solutions;
22) The "forward look" section, inviting suggested improvements; and
23) A final box providing a glossary of terms.

The section headed "web video" indicates whether a video-clip is available via a web link; this section is currently under development. The intention is to illustrate use of the test in field conditions in order to assist potential researchers. In the longer term, it is also intended to add links to other materials, such as schemas for protocols, Clinical Record Forms, and manuals of operation for given tests. It is hoped that Industry will consider this to be an opportunity to release nonsensitive, nonproprietary material for incorporation into the program.

IV. DEFINITION OF DRY EYE DISEASE

It was important for the Diagnostic Methodology Subcommittee to have a clear idea about the definition and classification of dry eye in order to put the tests presented into their proper context. As reported elsewhere in this supplement, the Definition and Classification committee has defined dry eye disease as follows:

> *Dry eye is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability, with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface.*[1]

Currently, ocular symptoms are included internationally within all definitions of dry eye, although it is acknowledged that asymptomatic patients exist who exhibit some of the objective features of dry eye and may be entitled to the diagnosis. The Japanese criteria were an exception to this,[2] but these criteria were revised in 2005 and are summarized in Appendix 4.

The issue of symptomatology in the diagnosis of dry eye is important, as one approach to the diagnosis of dry eye is based solely on the use of validated symptom questionnaires, whose administration, both in population studies and in the clinic, offer a highly accessible diagnostic instrument available to the comprehensive ophthalmologist and to the dry eye specialist alike.

V. CLASSIFICATION OF DRY EYE DISEASE

For its assignment, the Diagnostic Methodology Subcommittee regarded dry eye as a chronic, symptomatic ocular surface disease, which may, however, occasionally be asymptomatic. Asymptomatic dry eye implies that in the absence of symptoms, some objective criteria of dry eye may still be satisfied, such as tear hyperosmolarity, the presence of interpalpebral ocular surface staining, reduced tear production, or tear instability. The presence of symptoms may not always be clearcut, particularly when they develop insidiously. A patient may accept the development of irritative or visual symptoms as a matter of course (eg, as a normal part of aging), so that the symptoms are revealed only when a suitably structured questionnaire is applied.

Symptomatic ocular surface disease, (**SOSD**), is an umbrella term that includes:

1) Classical, *symptomatic dry eye*, as defined above, ie, patients experiencing the symptoms of dry eye and also exhibiting objective features of dry eye, however determined. In the current classification, this would include both *aqueous-deficient dry eye* (**ADDE**) and *evaporative dry eye* (**EDE**), as previously described[3]:

2) *Symptomatic lid disease*, including meibomian gland dysfunction (**MGD**) and anterior blepharitis, in the absence of dry eye;

3) *Symptomatic conjunctivitis and keratitis* (eg, allergic conjunctivitis, infective and noninfective keratitis and conjunctivitis) in the absence of dry eye.

The term *symptomatic ocular surface disease* has features in common with the term *dysfunctional tear syndrome* (**DTS**), a term coined by the Delphi group,[4] except that the term DTS was introduced as a *replacement* for the term dry eye, whereas, as discussed here, dry eye is seen as one component of SOSD. Any conceived form of SOSD can be expected to have its asymptomatic counterpart.

Dry eye is usually a symptomatic disorder that varies in severity and must be differentiated from other forms of SOSD. Severity ranges from a mildly irritative disorder of essentially nuisance value to the patient to a severely disabling disorder (eg, in Sjogren syndrome).[1] Although dry eye disease in its milder forms may respond to treatments that alleviate symptoms without modifying the disease process, recent pharmacological approaches are directed toward slowing, halting, or even reversing the disease process. Tests are therefore required that will discriminate between dry eye and its various subsets, identify precipitating factors, quantify disease severity, and demonstrate the effect of disease on a patients' quality of life.

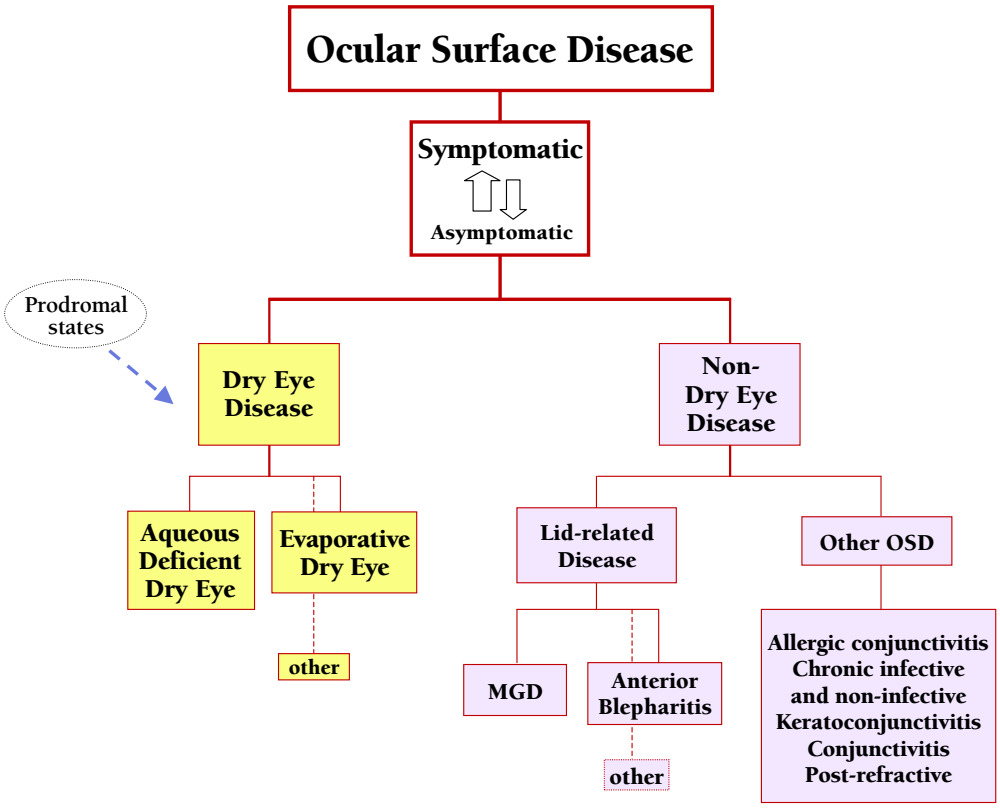

Figure 1. Schematic illustration of the relationship between dry eye and other forms of ocular surface disease. Ocular surface disease is either symptomatic or asymptomatic, but its various subgroups may coexist and interact. Therefore, a patient may suffer from both aqueous deficient and evaporative forms of dry eye, which will consequently be more severe than in the isolated disease. Also, dry eye may coexist with non-dry eye disease. (See text for further details; see also Chapter 1: Definition and Classification.[1]) OSD = Ocular surface disease; MGD = Meibomian gland dysfunction.

It is also necessary to distinguish dry eye disease from other SOSD. Any classification scheme should address the differential diagnosis of dry eye, such as MGD occurring on its own and disorders such as allergic eye disease, chronic non-dry eye conjunctivitis, and infective conjunctivitis and keratoconjunctivitis. Meibomian gland dysfunction and these other conditions may cause or contribute to dry eye, but exist in their own right as either symptomatic or asymptomatic disorders.

Other individuals should be recognized who are "at risk" of developing dry eye but show no evidence of disease. They are related to, but fall outside, the SOSD group, as they show no objective signs of any ocular surface damage that might constitute disease. An example would be those refractive surgery patients with reduced tear stability (eg, as assessed by the tear stability analysis system [**TSAS**]), who have greater risk of post-LASIK symptomatic keratitis and have a slower recovery time than those without a preoperative tear film instability.[5] Environmental factors may also contribute to risk.[1]

A general classification of ocular surface disease, including dry eye, is illustrated in Figure 1.

VI. TESTS USED TO DIAGNOSE AND MONITOR DRY EYE DISEASE

A. Uses of Tests

Tests are used for a variety of purposes:

1) To diagnose dry eye in everyday clinical practice.
2) To assess eligibility in a clinical trial (ie, recruitment). Such tests used in recruitment, may also be used as primary, secondary, or tertiary end points in a trial.
3) To follow quantitative changes over the duration of a clinical trial (monitoring). These tests might differ from those employed in recruitment. For instance, they might simply monitor the pharmacological action of a drug under study, eg, stimulation of mucin production.
4) To characterize dry eye as part of a clinical syndrome, eg, as in the harmonized classification criteria of Sjogren syndrome[6] (See Section VIII, Table 6).
5) To follow the natural history of the disorder. This opportunity is limited for dry eye, because treatment is so common in the population. However, the natural history of treated patients is also of interest, although they represent a heterogeneous population.

B. Shortcomings of Tests for Dry Eye

1. Selection Bias

No "gold standard" exists for the diagnosis of dry eye. Thus, when a test, eg, Schirmer test or rose bengal staining, is being evaluated for efficacy, the test population may have been classified as affected or non-affected based on those same tests. Similarly, the performance of any "new" test may be compromised when the test is assessed in a population of dry eye patients who have been diagnosed using unestablished criteria.

When studies of test efficacy look at how the test defines affected and unaffected individuals using individuals from the sample from which the diagnostic cut-offs were derived, this potentially results in a higher sensitivity and specificity rating than would have arisen from an independent sample. Also, because of the multi-factorial nature of dry eye, variable test efficacy is likely to occur from study to study.

2. Spectrum Bias

When the study sample consists of patients with either very mild or very severe disease, results are compromised because the severity of the disease in the sample studied has been highly selected.

Certain ground rules are proposed for appraising the performance of tests for dry eye diagnosis reported in the literature (Table 2).

1) Accept efficacy values on samples from which the test cut-off was derived (as is the case in most reports).
2) Exclude data from studies with selection bias due to the test being part of the original dry eye diagnostic criteria (to avoid study results with high, ie, false, sensitivity and specificity values).
3) To avoid spectrum bias, study samples should be large enough to include a range of dry eye patients with various etiologies.
4) The choice of the cut-off value for diagnosis and the test itself, unless there is some special physiological reason, should be based on a consideration of the relative consequences of having too many false-positives or too many false-negatives. Generally, in a screening test for a serious or life-threatening condition, it is desirable to have a test of high sensitivity (high detection rate)—with few false-negatives—since failure to detect the condition early can be fatal. In a mass screening test for a less serious condition or for one whose early detection is not critical, *it may be more desirable* to have a high specificity to avoid overburdening the health care delivery system with too many false-positives.
5) For dry eye screening tests, it is suggested that sensitivity and the predictive value of a positive test (**PPV**, see below) be maximized, ie, avoid high false-negative rates by "over-diagnosing" dry eye through choice of cut-off/test. This is appropriate when the patient is to be further assessed with other tests to finally diagnose dry eye. However, low false-negative rates (choice of test or cut-off maximize sensitivity) should be balanced by an acceptable PPV.
6) In diagnostic tests, optimize overall accuracy (**OA**) and combine this with a high sensitivity and PPV.
7) Simplify comparisons of screening and diagnostic tests by using single and simple terms for measuring test efficacy.

C. Appraisal of Tests Used for Screening

The purpose of screening is prevention, and it aims to identify people at high risk of a disorder. It is implicit in the screening process that a treatment is available that will reduce the morbidity of the disorder in a cost-effective manner. Screening has been defined, among persons who have not sought medical attention, as the "systematic application of a test or enquiry to identify individuals at sufficient risk of a…disorder to benefit from further investigation or…preventive action..."[26] It is implied that the disorder has serious consequences and that a remedy is available that could reduce morbidity.

Inclusion of symptoms within the definition of dry eye has an awkward implication in the context of screening. To identify those at risk of developing the disorder or who have unrecognized disease, screening is characteristically carried out on asymptomatic individuals who have not presented themselves for diagnosis; those who are symptomatic already have the disease. This "at-risk" group is likely to be represented by asymptomatic subjects whose pathophysiological background favors the development of dry eye. Perhaps, their lacrimal secretory level or their meibomian lipid secretion or delivery is at the lower limit of normal, so that with time they will pass into a state of insufficiency. They may have an unstable tear film, or they may be in the prodromal stages of a disease (eg, exhibiting nonophthalmic features of primary Sjogren syndrome), whose natural history dictates that they will eventually develop dry eyes. Members of this diverse group of subjects could be precipitated into dry eye by a number of biological, pharmacological or environmental events, ie, hormonal changes, drug exposure, high air or wind speeds, irritants, low humidity, and high temperatures. Exposure to such influences might engender dry eye symptoms in an at-risk group at a lower threshold than in subjects not at risk of dry eye disease.

At-risk subjects could be identified by "stress tests," some of which are included among the test templates that accompany this report and/or can be accessed at www.tearfilm.org. Whether or not such tests could or should become part of a "screening program" depends on whether any perceived therapeutic benefits would be economically justified. One such benefit might be to identify the suitability of individuals to work within a particular work environment, or to answer questions about the modifications of environments to avoid inducing symptomatic disease.

To be of value, a screening test should be simple, effective, applicable to a definable population, and cost-effective. In an effective screening program, a positive test ultimately leads to diagnostic tests, which, if positive, lead to timely

Table 2. Characteristics and current tests for dry eye

Test	Reference	Cut-off Value	Sensitivity (%)	FPR (%)	Specificity (%)	PPV*
Single Tests						
Questionnaires	†McMonnies[7]	Any	98	3	97	85
PRT	†Patel[8]	≤10mm	86	17	83	47
Rose Bengal	†Goren[9]	Any	25	10	90	31
Schirmer I	†Lucca[10]	<5mm/5min	25	10	90	31
Schirmer I	†Farris[11]	<3mm/5min	10	0	100	100
Schirmer I	†Bijsterveld[12]	<5.5mm/5min	85	17	83	47
Schirmer I	†Vitali[13]	<10mm/5min	83	32	68	31
F BUT	†Vitali[13]	<10s	72	38	62	25
NIBUT	†Mengher[14]	<10s	83	15	85	49
TMS-BUT	**†Goto[15]**	**<5s**	**98**	**37**	**63**	**32**
Evaporation Rate	†Khanal[16]	33 g/m^2/h	51	4	96	84
Meniscus Height	†Mainstone[17]	≤0.35mm	93	33	67	33
Meniscus Radius	**†Yokoi[18,19]**	**≤0.25mm**	**89**	**22**	**78**	**42**
Tear Film Index	†Xu[20]	≤95	67	40	60	23
Tear Turnover Rate	†Khanal[16]	12%/min	80	28	72	79
Osmolarity	†Farris[21]	>312 MOsm/L	95	6	94	73
Osmolarity	†Tomlinson[22]	>316 MOsm/L	69	8	92	60
Osmolarity	‡Tomlinson[22]	>316 MOsm/L	59	6	94	63
Osmolarity	†Tomlinson[22]	>312 MOsm/L	66	16	84	42
Osmolarity	†Tomlinson[22]	>322 MOsm/L	48	1	99	89
Osmolarity	†Khanal[16]	**317** MOsm/L	78	22	78	86
Osmolarity	†Sullivan B[23§]	>318MOsm/L	94	5	95	77
Lysozyme assay	**†van Bijsterveld[12]**	**dia <21.5mm**	**99**	**1**	**99**	**95**
Ferning	†Norn[24]	Area <0.06mm^2/µl	94	25	75	40
Lactoferrin	†Lucca[10]	<90	35	30	70	17
CombinedTests (Parallel)						
Sch + RB	†Farris[21]	Any/<1mm/min	77	51	49	21
Sch + BUT	†Farris[21]	<1mm/min/<105	78	44	56	24
Sch + BUT + RB	†Farris[21]	<1mm/min/<105/Any	80	51	49	22
TTR + Evap + Osmol	†Khanal[16]	<12%/>33/ >317	100	34	66	81
Combined Tests (Series)						
Sch + Osmol	**†Farris[21]**	**<1mm/min; >312**	**25**	**0**	**100**	**100**
Lacto + Osmol	**†Farris[21]**	**> 90; >312**	**35**	**0**	**100**	**100**
TTR + Evap + Osmol	†Khanal[16]	< 12%; >33; >317	38	0	100	100
Discriminant function						
Osmol + Evap + Lipid	†Craig[25]	< 0.4	96	13	87	56
TTR + Evap + Osmol	†Khanal[16]	> –0.4	93	12	88	58

The table shows the effectiveness of a range of tests, used singly or in combination, for the diagnosis of dry eye. The tests included in the table are those for which values of sensitivity and specificity are available in the literature. The predictive values of these tests (positive, negative and overall accuracy) are calculated for a 15% prevalence of dry eye in the study population. The data shown here is susceptible to bias; selection bias applies to those studies shown in dark shading, in these, the test measure was part of the original criteria defining the dry eye sample group and spectrum bias applied to those studies (shown in light shading) where the study population contained a large proportion of severe cases. Both of these forms of bias can lead to an artificially increased test sensitivity and specificity. In most of the studies listed above the efficacy of the test was shown for the data from the sample on which the cut off or referent value for diagnosis was derived (indicated by a †), again this can lead to increased sensitivity and specificity in diagnosis. Ideally test effectiveness should be obtained on an independent sample of patients, such data is shown in studies indicated by the symbol ‡.

Table 2 continues on following page

Table 2. Characteristics and current tests for dry eye *(continued)*

KEY to symbols and abbreviations used in Table 2.

*	Assumes a dry eye prevalence of 15% in the population studied.
†	Efficacy calculated in the sample from which the cutoffs were derived.
‡	Efficacy calculated in an independent sample of subjects.
§	Unpublished data

Definitions and Abbreviations

BUT	Tear break-up time
dia	Diameter of the disc observed with the radial-immuno-diffusion Lactoplate method
Evap	Tear film evaporation rate
F BUT	Fluorescein tear breakup time
FPR	False positive rate. The proportion of normals identified incorrectly as +ve by the test (Specificity is: 100-FPR)
Lacto	Lactoferrin assay using the Lactoplate method
NIBUT	Non-invasive tear breakup time
NPV	Predictive value of a negative test result
OA	Overall accuracy of test results
PPV	Positive Predictive Value: the probability of truly having dry eye among those with a positive test result
PRT	Phenol red thread test
RB	Rose Bengal staining
Selection bias	Bias built into an experiment by the method used to select the subjects who are to undergo treatment
Sensitivity	Detection rate: the proportion of patients with disease who have a positive test result
Specificity	Proportion of normal people with negative test result
Spectrum bias	Bias due to differences in the features of different populations eg, sex ratios, age, severity of disease, which influences the sensitivity and/or specificity of a test
TMS-BUT	Tear breakup time measured with the Topographic Modeling System[15]
TTR	Tear turnover rate, often measured with a scanning fluorophotometer (Fluorotron)

treatment. Where a series of tests is required to achieve a definitive diagnosis and initiate effective treatment, it is possible to assess the performance of the combination of tests. This may include a series of screening tests followed by one or more diagnostic tests, some of which may be performed simultaneously to save time.

The screening performance (efficacy) of a test can be estimated according to three parameters: 1) the *Detection Rate* (**DR**) or Sensitivity, 2) the *False-Positive Rate* (**FPR;** specificity is: 100-FPR), and 3) the ***O****dds of being* ***A****ffected in those with a* ***P****ositive test* ***R****esult* (**OAPR**). (This is the same as the PPV, if expressed as a probability.) Before a test is adopted, estimates of all three components should be available.

The relationship between affected and unaffected members of a population and the test result achieved can be represented in tabular form (Table 3).

The Detection Rate (DR) is the percentage of affected individuals who test positive. It is also referred to as the *sensitivity* of the test. The DR must be estimated using values from a continuous series of patients with the disorder, with no omissions.

$$DR = \frac{a}{a+c}$$

The False Positive Rate (FPR) is the percentage of unaffected individuals in a population who test positive. The FPR is usually estimated in a large series of apparently unaffected individuals.

$$FPR = \frac{b}{b+d}$$

The FPR, subtracted from 100, is also known as the *specificity* of the test.

The DR and FPR represent key characteristics of a test. Both are required for an assessment of its efficacy. The ideal test will have a high DR and a low FPR (ie, high specificity).

Table 3. Relationship between affected and unaffected members of population and test result achieved

		Presence of Disease				
		Yes	No	Sum		Population
Diagnostic Test Result	Positive +	a	b	a+b	=	total testing positive
	Negative –	c	d	c+d	=	total testing negative
Totals		a+c = total truly affected	b+d = total truly unaffected	a+b+c+d	=	total population

The DRs and FPRs for a number of tests used in dry eye diagnosis are presented in Table 2.

The third parameter is dependent on the prevalence of the disorder in the population studied. This is *The **O**dds of being **A**ffected in those with a **P**ositive test **R**esult* (OAPR [or PPV]). This is expressed as an odds value, eg, 1:3 or 1:100, etc. It can also be expressed as a percent probability (which in these cases would be: 1/4 × 100 = 25%, or 1/101 × 100 = 0.99%).

$$\text{OAPR} = \frac{a}{a+b}$$

D. Appraisal of Tests Used for Diagnosis

Diagnostic tests are applied to symptomatic or asymptomatic patients to obtain a diagnosis and, by inference, to exclude other diagnoses. A successful diagnosis can serve several functions, paramount of which is the opportunity for therapy. Therapy can ameliorate the symptoms of a disease, retard its progression, or produce a cure. Arrival at a successful diagnosis may also serve other functions, for instance, in relation to the natural history and prognosis of a disease, knowledge of which is of value to both patient and doctor. Also, a diagnosis, by excluding other diseases, may usefully indicate that a feared diagnosis is not present and that other kinds of therapy are not indicated.

1. Selecting a Cut-off Value

Test data may be qualitative (categorical, eg, with or without epiphora), semi-quantitative (ordinal, eg, grading by corneal staining), or quantitative (continuous, eg, the Schirmer test result in mm, intraocular pressure). For a test offering continuous data, it is appropriate to select a cut-off value to discriminate between affected and unaffected subjects. This may involve a trade-off between the DR and FPR, depending on the distribution of test values between these two groups. The DR and FPR are dependent on the selected cut-off values, and this is influenced by the overlap of values between affected and unaffected subjects. For instance, if there is no overlap in values between unaffected and affected subjects, then the cut-off will lie between the two data sets. However, where there is an overlap of values, which is usually the case, a cut-off must be chosen somewhere in the region of overlap.

The concept of choosing a cut-off is illustrated in the Figures 2a and 2b, which represent the situation in a hypothetical disorder in which the test variable is higher in the affected than in the unaffected population.[27] An example might be a staining score. When distributions are presented in this way, then the area to the right of the cut-off under the *unaffected* curve, provides the FPR, while the area to the right of the cut-off under the *affected* curve, gives the DR. Moving the cut-off to the right (as in Figure 2b) reduces the FPR but also reduces the DR.

2. The Likelihood Ratio

A useful way of expressing the interaction of DR and FPR is by calculating the *Likelihood Ratio* (**LR**), which is the ratio of those areas. The LR is a measure of the number of times individuals with positive results are more likely to have the disorder compared with individuals who have not been tested. A successful screening test might have an LR in the range of 5 to 25.

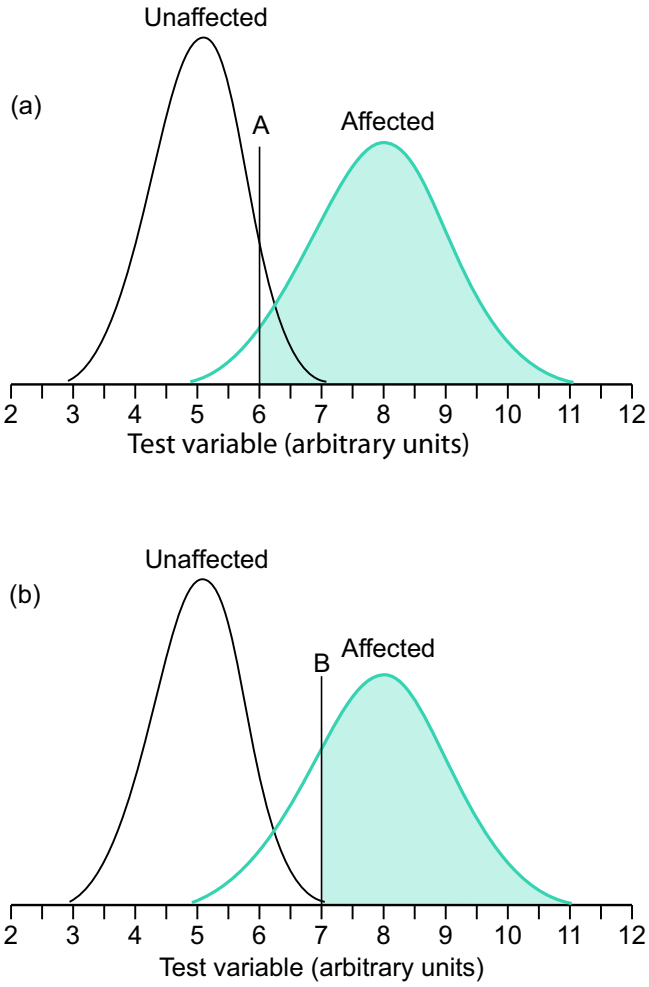

Figure 2. Illustrates how selection of the cutoff value influences the FPR and DR. See text for details.

3. Calculating the OAPR

The OAPR is a valuable parameter that represents the average chance of being affected for all individuals with a positive result by the test. It expresses the odds of the number of *true positives* to the number of *false positives*. For a given population, the OAPRs of different tests for the same condition may be compared directly with one another. There are two ways to calculate the OAPR (examples taken from Wald[26] and Wald and Cuckle[27]).

The first method uses a flow chart to estimate test performance.

Considering the total number of individuals identified as positive by a test within a defined population, a proportion will be true positives (determined by the DR of the test), and the remainder will be the false positives (determined by the FPR). The OAPR is the ratio of these two numbers, ie, OAPR = True Positives: False Positives.

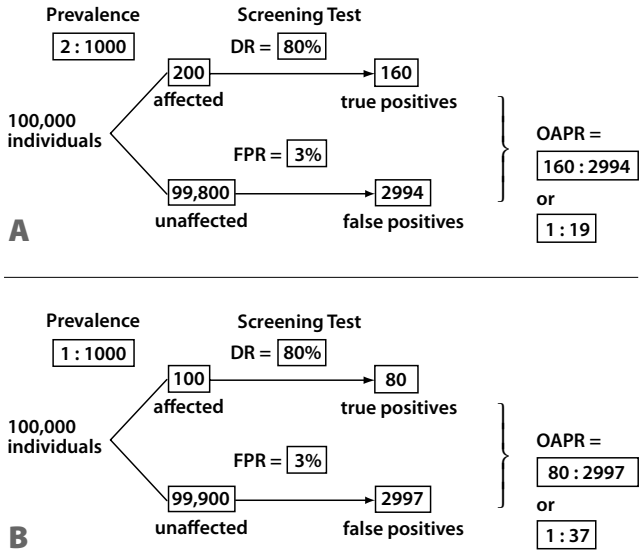

Figure 3. The influence of disease prevalence on the OAPR. See text for details.

Note that OAPR is influenced by the prevalence of the condition in the population studied.

If the test has a DR of 80% and an FPR of 3% then there are 160 true positives (80/100 x 200), and 2994 false positives (3/100 x 99,800) in the population. The OAPR can then be calculated as follows:

$$\text{OAPR} = \frac{\text{Number of true positives} = 160}{\text{Number of false positives} = 2994} = 1{:}19$$

The equivalent PPV is 5% [ie, 1/1+19 = 1/20 =5%] (Figure 3A).

With the same DR and FPR rates, but a prevalence of 1:1000, there are 100 affected and 99,900 unaffected.

In that case the test identifies 80 true positives and (3/100 x 99,900=) 2297 false positives, giving an OAPR that is twice that of the previous example:

$$\text{OAPR} = \frac{\text{Number of true positives} = 80}{\text{Number of false positives} = 2997} = 1{:}37$$

It can be seen that the OAPR falls as the prevalence falls (Figure 3B). The second method to calculate the OAPR uses the likelihood ratio. For a given population, the OAPR can be calculated by multiplying the LR by the prevalence of the disorder (expressed as an odds), ie, OAPR = LR x Prevalence as an odds [eg, 1:1000; 1:2000].

In the example given in Figure 4A, with a cut-off at 7, the DR is 80% and the FPR is 1%. In this case the LR is (80%/1%) = 80, and if the prevalence of the disorder is 1 per 1000 (ie, an odds of 1:999 or nearly the same as 1:1000), then:

$$\text{the OAPR} = 80 \times 1{:}1000 = 80{:}1000 = 1{:}1000 = 1{:}12.5$$

The two methods of calculating the OAPR are applicable to groups of subjects and are, therefore, of public health significance. However, it is also possible to calculate the OAPR for an *individual* with a particular positive result. This is illustrated in Figure 4B. In this situation, the LR for that individual is given by the height of *the affected population distribution curve* at the point of their test value, divided by the height of *the unaffected population distribution curve* at the same point. In the example given above, where the test value is 7 arbitrary units, the LR ratio is a/b = 12/1 = 12. Note that the vertical units are also arbitrary. Therefore, the OAPR for that individual is:

$$\text{OAPR} = \text{LR} \times \text{Prevalence as an odds [eg, 1:1000]} = 12 \times 1{:}1000 = 12{:}1000 = 1{:}1000/12 = 1{:}83.$$

This individual has a relatively low risk of being affected.

VII. A PROTOCOL FOR EVALUATING DRY EYE DIAGNOSTIC TESTS

The following protocol is suggested as a model for evaluating diagnostic tests for dry eye. It is proposed that:

1) The diagnostic test will be applied to a study sample of normal subjects and patients with dry eyes, as defined by symptoms, and the "traditional" ophthalmological tests, Schirmer I, tear film breakup time (**TBUT**), and ocular surface staining.

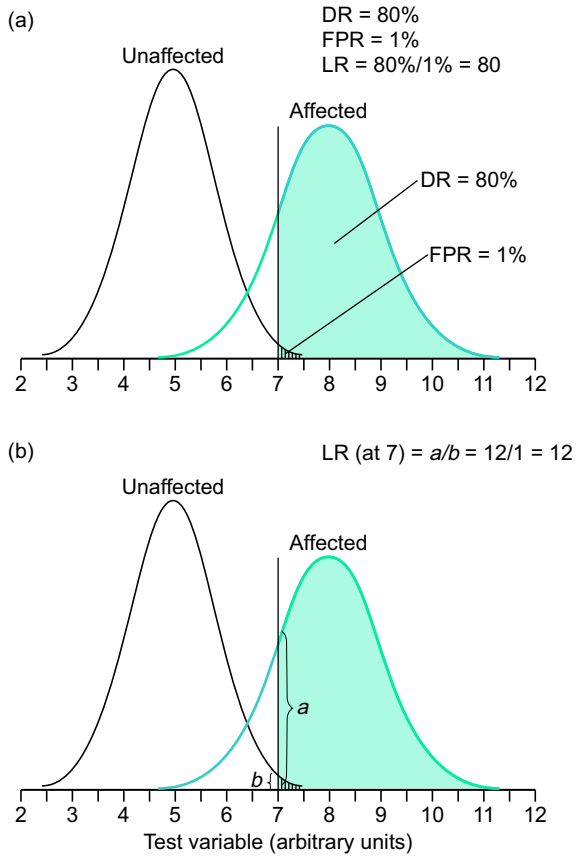

Figure 4. Calculation of the OAPR using the likelihood ratio. (a) For a group, (b) for an individual. See text for details.

2) The values obtained for the new diagnostic test in the two samples will be determined, frequency distributions of data will be compiled, and an initial cut-off value, distinguishing affected from non-affected, will be set at the intercept of the two frequency curves.

3) The sensitivity, specificity, and predictive values of a positive and negative test result and the overall accuracy of the test will be determined for this cut-off value.

4) A range of different cut-off values for the test statistic can then be analyzed by constructing a receiver-operator characteristic (**ROC**) curve to maximize the sensitivity and the specificity of the diagnostic test.

5) The proposed cut-off value thus determined for the test will then be assessed for its efficacy on a new, *independent sample* of normal and dry eye patients. An iterative process may then be required to arrive at a final cut-off value.

This approach should provide the best estimate of test performance.

Table 4A. A sequence of tests used in dry eye assessment, according to category

Group	Assessment	Technique
A	Clinical history	Questionnaire
	Symptoms eg, dry eye	Symptom questionnaire
B	Evaporation rate	Evaporimetry
C	Tear stability	Non-invasive TFBUT (or NIBUT)
	Tear lipid film thickness	Interferometry
	Tear meniscus radius/volume	Meniscometry
D	Osmolality; proteins lysozyme; lactoferrin	Tear sampling
E	Tear stability	Fluorescein BUT
	Ocular surface damage	Grading staining fluorescein; lissamine green
	Meniscus, height, volume	Meniscus slit profile
	Tear secretion turnover	Fluorimetry
F	Casual lid margin oil level	Meibometry
G	Index of tear volume	Phenol red thread test
H	Tear secretion	Schirmer I with anesthesia
	Tear secretion	Schirmer I without anesthesia
	"Reflex" tear secretion	Schirmer II (with nasal stimulation)
I	Signs of MGD	Lid (meibomian gland morphology)
J	Meibomian gland function	MG expression Expressibility of secretions Volume Quality
	Meibomian physicochemistry	Oil chemistry
K	Ocular surface damage	Rose bengal stain
L	Meibomian tissue mass	Meibography

From: Foulks G, Bron AJ. A clinical description of meibomian gland dysfunction. *Ocul Surf* 2003: 107-26.
Test invasiveness increases from A to L. Intervals should be left between tests. Tests selected depend on facilities, feasibility and operational factors.

VIII. RECOMMENDATIONS OF THE DIAGNOSTIC METHODOLOGY SUBCOMMITTEE: PREFERRED SCREENING AND DIAGNOSTIC TESTS FOR DRY EYE

The following recommendations are based on the commentary provided above and on the test data presented in Table 2. Readers are reminded that when a battery of tests is performed, these should be performed in the sequence that best preserves their integrity (Table 4). The tests discussed below are presented with this in mind.

A. Current Tests

For nearly half a century, a tetrad of diagnostic tests has been universally applied to assess symptoms, tear stability, ocular surface staining, and reflex tear flow.

1. Symptom Questionnaires

Over time, a number of symptom questionnaires have been developed for use in dry eye diagnosis, epidemiological studies, and randomized controlled trials (**RCT**s), which have received some psychometric or other validation and are available to practitioners for use in their clinics. The most important of these have been summarized elsewhere in this issue, where the necessity for reproducibility and the ability to measure severity and change ("responsive-

Table 4B. A practical sequence of tests

Clinical history
Symptom questionnaire
Fluorescein BUT
Ocular surface staining grading with fluorescein/yellow filter
Schirmer I test without anesthetic, or I with anesthetic, and/or Schirmer II with nasal stimulation
Lid and meibomian morphology
Meibomian expression
Other tests may be added according to availability

Further narrative information is provided in a template on the DEWS web site, entitled "A sequence of tests."
From Foulks G, Bron AJ. A clinical description of meibomian gland dysfunction. *Ocul Surf* 2003: 107-26.

ness") have been emphasized and templates presented.[28] According to their length and composition, such questionnaires explore different aspects of dry eye disease in varying depth, ranging from diagnosis alone, to the identification of precipitating factors and impact on quality of life. The time taken to administer a questionnaire may influence the choice of questionnaire for general clinical use, and, with this in mind, the number of questions administered in various questionnaires is listed in Table 5.

These questionnaires have been validated to differing extents, and they differ in the degree to which the dry eye symptoms assessed correlate with dry eye signs. For example such correlations were identified by the extensive Dry Eye Questionnaire (**DEQ**) of Begley et al,[34] but not by the questionnaire developed by Schein et al[30] or, to any great extent, in the study McCarty et al.[36]

The Diagnostic Methodology Subcommittee concluded that the administration of a structured questionnaire to patients presenting to a clinic provides an excellent opportunity for screening patients with potential dry eye disease. Clinic time can be used most efficiently by utilizing trained auxiliary staff to administer the questionnaires. Selection of a specific questionnaire will depend on practical factors, such as available staffing, and also the intended use of the data collected, eg, whether it will be used for diagnosis alone, recruitment to a clinical trial, or as a guide to treatment.[1]

Symptomatology questionnaires should be used in combination with objective clinical measures of dry eye status, as illustrated below.

Table 5. Symptom questionnaires in current use

Report	Questions administered	Reference
Womens' Health Study (WHS)	3	Schaumberg et al[29]
International Sjogren's Classification	3	Vitali et al[6]
Schein	6	Schein et al[30]
McMonnies	12	McMonnies and Ho[31]
OSDI	12	Schiffman et al[32]
CANDEES	13	Doughty et al[33]
Dry Eye Questionnaire (DEQ)	21	Begley et al[34]
IDEEL (3 modules, 6 scales)	57	Rajagopalan et al[35]

2. Grading Ocular Surface Staining

In clinical trials in some countries, it is current practice to grade staining of the cornea using fluorescein dye and to grade staining of the conjunctiva using lissamine green. This is done for reasons of visibility and is discussed in detail elsewhere.[37] It is, however, possible to detect and score staining on both the cornea and conjunctiva together, using fluorescein alone, if fluorescence is viewed through a yellow barrier filter (eg, Wratten 12).[38]

Three systems for quantifying staining of the ocular surface are in current use, the van Bijsterveld system,[12] the Oxford system,[37] and a standardized version of the NEI/Industry Workshop system,[3]—for instance, the version developed for the CLEK study and used in the assessment of clinical methods for diagnosing dry eye (Appendices 5 and 6).[38] The Oxford and CLEK systems use a wider range of scores than the van Bijsterveld system, allowing for the detection of smaller steps of change in a clinical trial. The CLEK system, which assesses several zones of the cornea, has the advantage of scoring staining over the visual axis, providing the opportunity to relate surface changes to changes in visual function. No studies have been published that indicate that one grading system is innately better than another, but interconversion of the van Bijsterveld and Oxford scores has been estimated in an unpublished comparative study (J. Smith, personal communication).

Selection of a diagnostic cut-off for recruitment to a clinical trial is influenced by the need to identify a score that is sufficiently high to be able to demonstrate a response to treatment, but is sufficiently low to permit the recruitment of adequate numbers. Some workers have used a van Bijsterveld cut-off of ≥ 3 in recruiting dry eye patients for clinical studies. For dry eye diagnosis within the framework of Sjogren syndrome, a cut-off of ≥ 4 was derived by the American-European consensus group in a large multicenter study. [6]

3. Tear Film Stability—Tear Film Break-Up Time (TFBUT)

Details of test performance are given in Appendix 7, including the need for application of a standard volume of fluorescein and the use of a yellow barrier filter to enhance the visibility of the breakup of the fluorescent tear film. The established TFBUT cut-off for dry eye diagnosis has been < 10 seconds since the report of Lemp and Hamill in 1973.[39] More recently, values lying between ≤ 5 and < 10 seconds have been adopted by several authors, possibly based upon the 2002 report of Abelson et al,[40] which suggested that the diagnostic cut-off falls to < 5 seconds when small volumes of fluorescein are instilled in the conduct of the test (eg, using 5μl of 2.0% fluorescein in that study—many clinical trials adopt the practice of pipetting small, fixed volumes of dye). At present, sensitivity and specificity data to support this choice have not been provided, and the population in that study has not yet been defined. Refinement of this kind of data would comprise a welcome addition to the literature. Selecting a cut off below < 10 seconds will tend to decrease the sensitivity of the test and increase its specificity.

4. Reflex Tear Flow—the Schirmer Test

The Schirmer test score (length of wetting after 5 minutes) is commonly treated as a continuous variable, but it

is more properly termed a pseudocontinuous variable, as wetting length values are generally taken as the nearest integer or half integer rather than as continuous fractions of a millimeter.

The Schirmer test without anesthesia is a well-standardized test that is currently performed with the patient's eyes closed (Appendix 8).[6] There is wide intrasubject, day-to-day, and visit-to-visit variation, but the variation and the absolute value decrease in aqueous-deficient dry eye, probably because of the decreased reflex response with lacrimal failure. The diagnostic cut-off employed in the past was ≤ 5.5 mm in 5 minutes, based on the van Bijsterveld study,[12,41] and the studies of Pflugfelder et al[42,43] and others[6] have made a case for using ≤ 5 mm. More recently, many authors and clinical trialists have adopted a cut-off of < 5 mm although the basis for this shift is unclear. Lowering the cut-off decreases the detection rate (sensitivity) but increases the specificity of the test. The van Bijsterveld study, although a model study in many ways, suffered from selection bias and, therefore, a refinement of this value, using appropriate studies, is needed (see above). In the meantime, it is reasonable to carry out the Schirmer test using a cut-off of ≤ 5 mm in 5 minutes.

Table 6. Revised international classification criteria for ocular manifestations of Sjogren syndrome

I. Ocular symptoms: a positive response to at least one of the following questions:
1. Have you had daily, persistent, troublesome dry eyes for more than 3 months?
2. Do you have a recurrent sensation of sand or gravel in the eyes?
3. Do you use tear substitutes more than 3 times a day?

II. Oral symptoms: a positive response to at least one of the following questions:
1. Have you had a daily feeling of dry mouth for more than 3 months?
2. Have you had recurrently or persistently swollen salivary glands as an adult?
3. Do you frequently drink liquids to aid in swallowing dry food?

III. Ocular signs: that is, objective evidence of ocular involvement defined as a positive result for at least one of the following two tests:
1. Schirmer's I test, performed without anaesthesia (≤5 mm in 5 minutes)
2. Rose bengal score or other ocular dye score (≥4 according to van Bijsterveld's scoring system)

IV. Histopathology: In minor salivary glands (obtained through normal-appearing mucosa) focal lymphocytic sialoadenitis, evaluated by an expert histopathologist, with a focus score ≥1, defined as a number of lymphocytic foci (which are adjacent to normal-appearing mucous acini and contain more than 50 lymphocytes) per 4 mm^2 of glandular tissue

V. Salivary gland involvement: objective evidence of salivary gland involvement defined by a positive result for at least one of the following diagnostic tests:
1. Unstimulated whole salivary flow (≤1.5 ml in 15 minutes)
2. Parotid sialography showing the presence of diffuse sialectasias (punctate, cavitary or destructive pattern), without evidence of obstruction in the major ducts
3. Salivary scintigraphy showing delayed uptake, reduced concentration and/or delayed excretion of tracer

VI. Autoantibodies: presence in the serum of the following autoantibodies:
1. Antibodies to Ro(SSA) or La(SSB) antigens, or both

Reprinted with permission from: Vitali C, Bombardieri S, Jonnson R, et al. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002;1:554-8.

5. Tear Osmolarity

The place of tear osmolarity measurement in dry eye diagnosis is well established, and its adoption has several attractions. There is considerable value in assessing a parameter that is directly involved in the mechanism of dry eye. Tear hyperosmolarity may reasonably be regarded as the signature feature that characterizes the condition of "ocular surface dryness."[1] Furthermore, in several studies, as illustrated in Table 2, development of a diagnostic osmolar cut-off value has utilized appropriate methodology, using an independent sample of dry eye patients. Thus, the recommended cut-off value of 316 mOsm/l can be said to be well validated.[22]

In the past, although the measurement of tear osmolarity has been offered as a "gold standard" in dry eye diagnosis,[11] its general utility as a test has been hindered by the need for expert technical support; thus, its use has been confined to a small number of specialized laboratories. The feasibility of this objective test is greatly enhanced by the imminent availability of a commercial device that will make the technology generally available (see below).[23,45]

6. Combined Tests in Current Use

In various RCT settings, different authors have adopted different approaches to the recruitment of dry eye patients, on an *ad hoc* basis, usually requiring subjects to satisfy entry criteria including a symptom or symptoms together with one or more positive signs (eg, a positive TFBUT test, staining grade, or Schirmer test).

The best example of the validated use of a combination of tests in dry eye for diagnosis is provided by the classification criteria of the American-European consensus group.[6] These criteria require evidence for a single ocular symptom and a single ocular sign for the diagnosis of dry eye as a component of Sjogren syndrome, as summarized in Table (Table 6).

B. Future Tests

Looking to the future and based on the currently available data (Table 2), the use of various tests, singly or in combination, can be considered as adjunctive approaches to dry eye screening and diagnosis. They are summarized briefly below:

1. Screening Tests for Dry Eye Disease

Screening tests should maximize sensitivity and "dry eye overdiagnosis." Such tests include single measures of

meniscus height (using appropriate technology), tear ferning; or parallel combinations of tear turnover rate (**TTR**) + evaporation + osmolarity, or weighted combinations (by discriminant function analysis) of osmolarity + evaporation + lipid classification or TTR .

Because a screening test should be rapid and simple, the preference might be for a meniscus height or radius measure.

2. Diagnostic Tests for Dry Eye Disease

Diagnostic tests should combine high overall accuracy with good sensitivity. As noted above, the measurement of tear osmolarity may turn out to be the single most important, objective test in the diagnosis of dry eye disease. Alternative candidates as objective tests include 1) the parallel combination of TTR + evaporation + osmolarity, or the weighted combination (by discriminant function analysis) of osmolarity + evaporation + lipid classification or TTR.

The most effective test candidates are complex and not easily applicable, clinically. This might suggest noninvasive TFBUT as the clinical alternative.

Certain combinations of dry eye-related tests have been used to predict the risk of contact lens intolerance in patients presenting for fitting with hydrogel contact lenses.[1,44]

Table 7. A selected list of some emerging technologies

Invasiveness	Comment	Reference
Non-invasive	Symptom questionnaires (also see Table 2)	
	Schein	Schein et al[30]
	OSDI	Schiffman et al[32]
	DEQ	Begley et al[34]
	IDEEL	Rajagopalan et al[35]
	Utility assessment	Buchholz et al[45]
Non- to Minimal	*Optical sampling*	
	Meniscometry (Appendix 10)	Yokoi et al[46]
	Lipid layer interferometry (Appendix 11)	Yokoi et al[47]
	Tear stability analysis system (Appendix 12)	Kojima et al[48]
	High speed video—tear film dynamics	Nemeth et al[49]
	OCT tear film and tear film imaging	Wang et al[50]
	Confocal microscopy	Erdelyi[51]
	Tear fluid sampling	
	Strip meniscometry	Dogru et al[52]
	Sampling for proteomic analysis	Grus et al[53]
	Osmolarity eg, OcuSense (Appendix 9)	Sullivan[54]
Moderate	Meibomian sampling; Meibometry (Appendix 13)	Yokoi et al[55]
	Meibography (Appendix 14)	Mathers, et al[56]
Invasive non-stress	Staining: new dyes Digital photography of surface staining	Note: These techniques may reflect steady state conditions at the time of sampling, even though they disturb the steady state with respect to downstream tests.
	Impression and brush cytology—coupled to flow cytometry (Appendices 15 and 16)	
	Lacrimal scintigraphy	
Stress Tests	Functional visual acuity	Ishida et al[57]
	Controlled Adverse Environment (CAE)	Ousler et al[58]
	S-TBUD (Areal BUT while staring)	Liu et al[59]
	Forceful blink test (Korb)	Korb[60]

DEQ = Dry Eye Questionnaire; IDEEL=Impact of Dry Eye on Everyday Life; OCT =Ocular Coherence Tomography; OSDI =Ocular Surface Disease Index; S-TBUD=Staring Tear Breakup Dynamics.

C. Emerging Technologies

The purpose of this section is to review those diagnostic technologies that show promise for advancing our ability to investigate, monitor, or diagnose dry eye disease in the future. Many of these technologies are described within the web-based diagnostic test templates, and some are at a nascent stage. Such tests start life as prototype instruments that are used by investigators within a research environment. Some of these never see broader application as inexpensive, easy-to-use tools that can be used in the clinical setting. There is particular interest is in those technologies that might be adapted and adopted for everyday clinical use. The tests discussed here are summarized in Table 7. The new technologies are at various stages of development. Some are elaborations of old technologies and some are entirely new.

Most technologies sample the eye in some fashion, and it is useful to consider whether that sampling process is noninvasive, minimally invasive, or invasive. In tear sampling, a non- or minimally-invasive technique has the major advantage that it captures data from the surface of the eye without significantly inducing reflex tearing. Reflex tearing has been a major obstacle to the interpretation of aqueous tear-sourced data from the earliest days of tear research.

There are evident advantages to the capturing of data that represent the steady state, whether these are physiological data or pathologic data.

The problem of reflex tearing has, of course, greatly influenced the interpretation of tear compositional data. For this reason, techniques that gather information from the tear film by processing reflected light or images from the tear film surface have a particular attraction as representing the "true" state of the ocular surface. This would include techniques such as interferometry, meniscometry, high-speed videotopography and optical coherence tomography (**OCT**). Some of these techniques offer the opportunity of delivering on-line data to a data capture system, allowing processing of the dynamic behavior of the tear film. In the same way, the capturing of images of cells and other materials at the ocular surface on-line seems to represent an opportunity to view the steady state.

It is the view of the Diagnostic Methodology Subcommittee that access to the steady-state presents less of a sampling problem when data are directly acquired from the ocular surface (eg, sampling cells or mucin from the ocular surface by impression cytology or brush cytology), as the sample makes an instantaneous statement about the steady state. Here, however, there may be problems in interpreting the sample because of the variable and partial nature of the sampling procedure. These problems can be handled in part by standardization. Also, although such sampling may take a "snapshot" of the steady state, such procedures (ie, impression cytology), because they are invasive, will influence subsequent sampling events. Therefore, they may need to be placed at the end of a series of tests.

It is our expectation that the sampling of expressed meibomian lipid is likely to reflect the steady state condition of the meibomian glands at the time of collection. Here we encounter other kinds of difficulties; for instance, the expressed material is all presecretory and, therefore, it does not fully reflect the nature of lipids delivered to the tear film, and in the case of meibomian gland dysfunction, the expressed material is likely to be increasingly contaminated with keratinized epithelial debris. For this reason, many publications refer to this expressed material as "meibomian excreta" or "meibum." Nonetheless, such expressed material, whether secretion or excreta, is likely to reflect the steady state of the meibomian and ductular product.

In summary, the Diagnostic Methodology Subcommittee concludes that in studying the ocular surface, there is a reasonable opportunity to obtain steady-state information about ocular surface cells and the meibomian gland and duct status. For studying the tear film, the greatest opportunity lies in the use of noninvasive techniques involving the sampling of optical radiation reflected from the tear film. However, even with noninvasive techniques, we must be cautious, as a gradual change has been observed in meniscus curvature by meniscometry in subjects sitting in apparently stable room conditions over a matter of several minutes, suggesting that it is very easy to induce minor degrees of reflex tearing under "test" conditions. Consequently, such techniques hover in a gray zone between non- and minimally-invasive in character. On the other hand, we anticipate that the designation of "minimally invasive" may be reasonably applied to direct sampling of tears under circumstances where sample volumes are in the low nanolitre range. This relates to sampling for proteomic analysis and to the depression of freezing point and "lab-on a-chip" methods for estimating tear osmolarity.

In considering noninvasiveness, it is important to note that there have been significant advances in the development of questionnaires to diagnose dry eye, identify precipitating or risk factors and explore quality-of-life implications. Nonetheless, even questionnaires are not truly non-invasive, since whenever people are observed within a study, their behavior or performance is altered (the "Hawthorne effect"[61]).

Although emerging technologies have focused on the development of noninvasive techniques to observe the steady state conditions of dry eye, there is one area where the invasive test plays a useful role. This relates to various stress tests for dry eye diagnosis, which aim to subject the eye to some sort of stress that will reveal a predisposition to dry eye. Such stress tests include the staring tear breakup dynamics (**S-TBUD**) test, forced closure test, and use of a controlled adverse environment (**CAE**).

In general, the recommended approach favors technologies that allow changes in tears at the ocular surface to be detected while causing the least disturbance to tear film dynamics during sampling. Proteomic and related techniques are examples of these. Such non- or minimally-invasive technologies offer improved acceptability to the patient and the possibility of assessment at something close to the steady-state. In addition to disturbing the tear film and altering the accuracy of the test, an invasive test is more likely to influence the outcome of another test performed sequentially, perhaps as part of a battery of tests. Some minimally invasive technologies are already in place and require only further refinement, such as the development of micro-processor-controlled systems to capture and represent data. In other technologies, the induction of reflex tearing at the time of tear sampling still exists as a problem to be overcome.

IX. SUMMARY OF RECOMMENDATIONS

A. Diagnosis of Dry Eye Disease

Two factors influence our recommendations of diagnostic tests for dry eye. First, many candidate tests derive from studies that were subject to various forms of bias (Table 2). This means that the cut-offs that they propose may be unreliable. Second, several tests with excellent credentials are not available outside of specialist clinics. We therefore offer here a pragmatic approach to the diagnosis of dry eye disease based on the quality of tests currently available and their practicality in a general clinic, but we ask readers to apprise themselves of the credentials of each test by referring to Table 2.

1) Seven sets of validated questionnaires, of differing

length, are listed in Table 5 (refer to the website, www.tearfilm.org, and the report of the Epidemiology Subcommittee[28] for further details). We recommend that practitioners adopt one of these for routine screening in their clinics, keeping in mind the qualitative differences between the tests.

2) The dry eye component of the international classification criteria for Sjogren syndrome requires one ocular symptom (out of three) and one ocular sign (out of two) to be satisfied (Table 6).[6]
3) Tear Evaluation

a) Tear osmolarity: Although techniques to measure tear osmolarity are currently inaccessible to most practitioners, the development of commercial instruments may make such measurements feasible in the near future. As an objective measure of dry eye, hyperosmolarity is attractive as a signature feature, characterizing dryness. A number of studies, including the study of an independent sample, suggest a diagnostic cut-off of ≥ 316 MOsm/L.

b) Non-invasive TFBUT: If the studies shown in Table 2 that are potentially susceptible to selection or spectrum bias are ignored, the simple clinical alternative for dry eye diagnosis might be noninvasive TFBUT measurements that give moderately high sensitivity (83%) with good overall accuracy (85%).

c) Tear function: The tear function index (**TFI**) has been used in the diagnosis of dry eye as a component of Sjogren syndrome. It is the quotient of the Schirmer value and the tear clearance rate, and a standard kit is available (see web template). The sensitivity of the test is cited as 100% with a cut off of < 40.[62]

4) Better test performance can be achieved when tests are used in combination, either in series or in parallel and the opportunity should be taken to review some of the standard tests cited above, using large, independent populations of subjects.

B. Monitoring Dry Eye Disease

Many of the tests used to diagnose dry eye are also used to monitor its progress, either in the clinic or within clinical trials. Additional tests, many of them referred to in this DEWS Report or presented on the website (www.tearfilm.org) can be used to follow the progress of the disease. In the future, these may include increasingly sophisticated techniques applied to tiny tear volumes with minimal invasiveness. Such tests will help to identify important changes in the native and inflammatory components of the tears in dry eye.

X. SUMMARY AND CONCLUSIONS

The purpose of this report was to review the literature and develop a resource of tests used in dry eye disease diagnosis and monitoring. These are displayed as templates on the TFOS website (www.tearfilm.org), which will be updated from time to time. A selection is presented herein. To give guidance as to their selection and interpretation, we have indicated some of their shortcomings and sources of bias. Our aim has been to facilitate standardization and validation. In general, with some exceptions, there is still a deficiency of symptom questionnaires and objective tests that have been adequately validated within well-defined sample populations. These deficiencies are remediable and will be a stimulus for future research. As we emphasize here, in considering emerging technologies, the way forward will be with new, minimally invasive techniques that sample the eye and preserve its steady state.

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APPENDIX 1. ALPHABETICAL LISTING OF TESTS USED TO DIAGNOSE AND MONITOR DRY EYE

Allergy conjunctival eosinophils
Allergy conjunctival provocation test
Allergy tear IGE
• • •
Basal tear volume
Brush cytology
• • •
CCLRU—Hyperemia and other grading scales
Conjunctivochalasis
• • •
Fluorescein permeability
Flow cytometry
• • •
Endocrine markers report
EQ-SD (questionnaire)
• • •
Ferning
Forceful blink test
Functional visual acuity
• • •
Grading staining—Nichols CLEK B
Grading staining—Oxford scheme
Grading staining—van Bijsterveld
• • •
Hamano thread test
• • •
Impression cytology
• • •
Lacrimal biopsy
Lid margin disease criteria
LASIK-induced Neuro-Epitheliopathy (LINE)
• • •
Meibography
Meibomian gland expression
Meibomian lipid analysis
Meibomian lipid sampling
Meibomian microbiology
• • •
NIBUT
• • •
Ocular Protection Index (OPI)
Osmolarity OcuSense overview
Osmolarity—Depression of freezing point
Osmolarity OcuSense—Sullivan
Osmolarity—Vapor pressure
• • •
Rheumatic criteria
• • •
SBUT
Schirmer I European criteria 1994
Schirmer I Farris
Schirmer I Nichols
Schirmer I van Bijsterveld
Schirmer Pflugfelder A
Schirmer Pflugfelder B
Scintigraphy
SF-36
Sicca index
Sjogren syndrome—Direct sialometry
Sjogren syndrome—Salivary-scintigraphy
Sjogren syndrome—Sialography
Sjogren syndrome—Hematology
Sjogren Serology—Martin
SSI (Sjogren Syndrome Index)—Bowman
Symptoms DEQ (questionnaire)
Symptoms IDEEL (questionnaire)
Symptoms McCarty (questionnaire)
Symptoms McMonnies (questionnaire)
Symptoms NEI-VFQ25 (questionnaire)
Symptoms OSDI (questionnaire)
Symptoms Schein (questionnaire)
Staining exam form-1 from Nichols
• • •
TBUD
Tear evaporation
Tear flow fluorimetry
Tear lipid interferometry
Tear meniscus height
Tear meniscus radius
Tear protein profiles
Tear Stability Analysis System (TSAS)
Tear turnover fluorimetry
Tear volume fluorimetry
Tests used in combination
Combined tests—Afonso 1999
Combined tests—Bjerrum 1997
Combined tests—European criteria 1994
Combined tests—Nichols 2004
Combined tests—Pflugfelder 1998
Combined tests—Shimazaki 1998
Combined tests—van Bijsterveld 1969
Tear film breakup time (TFBUT)
Thermography
Time-trade-off approaches to dry eye severity

APPENDIX 2. FUNCTIONAL GROUPINGS OF TESTS USED IN THE ASSESSMENT OF DRY EYE

- **1. Symptoms tests**
  - *Questionnaires*
    - NEI-VFQ25
    - McMonnies
    - Schein
    - McCarty
    - OSDI
    - DEQ
    - IDEEL
  - ***Visual function***
    - LogMar acuity
    - Contrast sensitivity
    - Functional visual acuity
- **2. Aqueous tears**
  - ***Tear volume***
    - Fluorimetry
    - Hamano thread
    - Periotron test—"basal tear volume"
  - ***Tear meniscus***
    - Radius of curvature
    - Height
    - Area of cross-section
  - ***Tear film thickness***
  - ***Tear flow***
    - Fluoroimetry
    - Schirmer test
      - Schirmer I
      - Dynamic Schirmer
      - Schirmer II
      - Reflex Schirmer
  - ***Tear turnover***
    - Dye dilution
    - Tear clearance
    - Fluorimetry
  - ***Tear evaporation***
    - Evaporimetry
- **3. Tear stability and visual function**
  - ***Visual acuity***
    - ETDRS
    - Functional visual acuity
  - ***Tear stability***
    - Breakup time (BUT)
    - SBUT: Symptomatic BUT
    - Tear film BUT fluorescein
    - Noninvasive BUT (NIBUT)
    - Tear thinning time
    - Topographic analysis
    - Tear stability analysis system
    - Wavefront analysis
- **4. Tear composition**
  - ***Biological fluids***
    - Aqueous tears
      - Lactoferrin
      - Lysozyme
      - Peroxidase
      - Immunoglobulin A
      - Ceruloplasmin
      - Inflammatory mediators
      - Matrix metalloproteinases
      - Other proteins
      - Mucins
      - Lipids
  - ***Cells in biofluids***
    - Inflammatory cells
      - Epithelial cells
      - Tear debris
  - ***Surface cells***
    - Impression cytology
    - Flow cytometry
    - Brush cytology
    - Confocal microscopy
  - ***Meibomian lipids***
    - Evaporimetry
    - Interferometry
    - Thickness
    - Grading
    - Meibometry
    - Meibography
    - Morphology in MGD
    - Expressed oil quality
    - Lipid chemistry
  - ***Tears: physical***
    - Osmolarity
      - Depression of freezing point
      - Vapor pressure osmometry
      - Conductivity OcuSense
      - Electrolyte composition
    - Tear ferning
  - ***Surface damage***
    - Grading staining
    - Fluorescein stain
    - Rose Bengal stain
    - Lissamine green
    - Double staining
- **5. Other criteria**
  - Tear function index (TFI)
  - Ocular protection index (OPI)
  - Conjunctivochalasis score
  - Blink characteristics
  - Distinction from allergy
  - Lid margin disease criteria
  - Microbiology and lid disease
- **6. Sjogren syndrome**
  - Serological tests
    - Anti-Ro
    - Anti-La
    - Anti-M3 receptor
    - Anti-fodrin
  - Minor salivary gland biopsy
  - Lacrimal gland biopsy
  - Systemic endocrine findings
  - Tests of salivary function
    - Biscuit test
    - Sialography
- **7. Tests for assorted disorders**
  - Wegener's: Positive ANCA
  - Rheumatoid arthritis: Positive Rh-F
  - Systemic lupus erythematosus
  - LASIK-Induced Neuro Epitheliopathy

APPENDIX 3. A PROFORMA DIAGNOSTIC TEMPLATE

DEWS	**DRY EYE: DIAGNOSTIC TEST TEMPLATE**	
RAPPORTEUR	Please insert your name	Date:DD/MM/YY
REVIEWERS	Names of additional reviewers added here	
NAME OF TEST	eg, **Schirmer 1**	
TO DIAGNOSE	*Test used to diagnose — eg, aqueous tear deficiency (ATD).*	REFERENCES
VERSION of TEST	[V] *Please call your preferred version, version 1. Other versions should be submitted on separate templates and numbered, not necessarily in priority order.*	*Please reference the source of this version.*
DESCRIPTION	*This should be a one or two line statement saying what the test is for.*	
NATURE of STUDY	*If you wish to refer to a specific study in detail, enter the details here.*	
CONDUCT of TEST	*Please describe all steps of the test in sufficient detail to provide a template for a trainer.*	
Results of Study	*If you have described a specific study in detail, place the results here.*	
Web Video	Available [ ] *If instruction would be aided by a video of the technique, please tick this video box.*	
Materials	*Please list the nature and sources of materials used for the test as described.*	
Variations of Technique		
Standardization	Time of day: [ ] Temperature: [ ] Humidity: [ ] Air speed: [ ] Illumination: [ ] Other: [ ] *Tick the boxes if you think that such standardization would improve the repeatability of the test.*	
Diagnostic Value	This version: [ ] Other version: [ ] *Please state if these stats relate to this version or another cited version.* *Please cite statistics indicating the diagnostic value of the test in a referenced study.*	*Please cite reference to stats used*
Repeatability	Intra-observer agreement: [ ] Inter-observer agreement: [ ]	
Sensitivity	(true positives): [ ]	
Specificity	(100 – false positives): [ ]	
Other Stats	*If you have other stats for this or related versions of the test, add as many rows as necessary and cite the reference.*	
Level of Evidence		
Test Problems	*Is there a problem with this test?*	
Test Solutions	*Can you suggest an improvement?*	
Forward Look	*What future developments do you foresee?*	
Glossary	*Please explain abbreviations*	

REFERENCES
[*To be inserted*]

APPENDIX 4. A NOTE ON THE JAPANESE CRITERIA FOR DRY EYE DIAGNOSIS

The previous Japanese dry eye diagnostic criteria were revised by the Japanese Dry Eye Research Society after the 1994-95 NEI/Industry workshop (Miyawaki S, Nishiyama S. Classification criteria for Sjogren's syndrome—sensitivity and specificity of criteria of the Japanese Ministry of Health and Welfare (1977) and criteria of European community (1993). *Nippon Rinsho* 1995;53:2371-5). The criteria, unpublished in the English literature, omitted symptoms from the diagnostic criteria at that time, because objective and subjective findings did not appear to correlate. Following the DEWS meeting of 2004, the importance of symptoms was accepted in Japan and the criteria have been modified.

The Japanese criteria prior to the 2004 DEWS meeting were:

1) Qualitative or quantitative disturbance of the tear film (quantity: Schirmer test less than 5 mm or phenol red thread test less than 10 mm; quality: BUT less than 5 sec)
2) Conjunctivocorneal epithelial damage (excluding all other etiologies other than that listed under number 1)
   Fluorescein staining greater than 1 point
   RB staining greater than 3 points
   (The presence of either fluorescein or RB staining is finding sufficient to satisfy criterion number 2)

The presence of both 1 and 2 = Definite dry eye. Presence of 1 or 2 = Probable dry eye

The Japanese diagnostic criteria have been revised by the Japan Dry Eye Research Society in August 2005, to include symptoms, as follows.

New Diagnostic Criteria of the Japan Dry Eye Research Society: Revised in August 2005

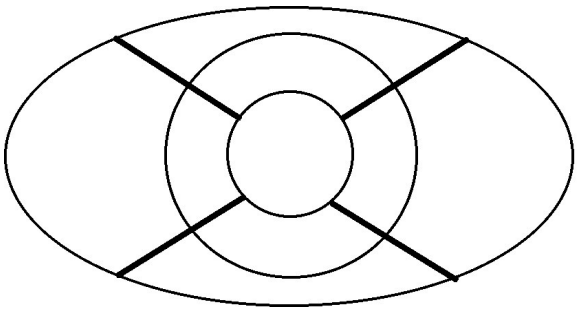
	Definite DE		Probable DE	
Symptoms	Yes	Yes	Yes	No
Tear film quality/quantity—disturbed	Yes	No	Yes	Yes
Epithelial damage	Yes	Yes	No	Yes

The phenol red thread test has been removed from the diagnostic criteria.
A fluorescein staining score of above 3 points is now required as positive staining (instead of 1 point).

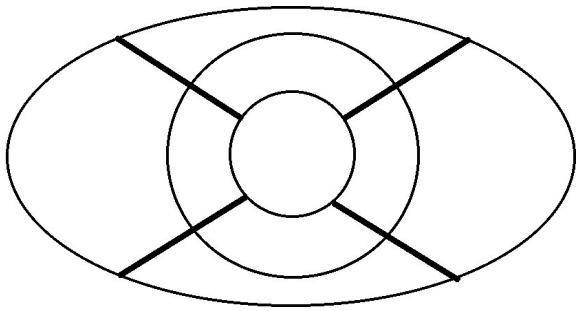
APPENDIX 5

DEWS	**DRY EYE: DIAGNOSTIC TEST TEMPLATE**	
RAPPORTEUR	A.J. Bron	22nd Oct 2004
TEST	**GRADING STAINING: CLEK Schema**	
TO DIAGNOSE	The scheme is used to estimate surface damage in dry eye.	REFERENCES
VERSION of TEST	[V1] [CLEK study]	Barr et al 1999 Lemp 1995
DESCRIPTION	Surface damage to the exposed eye, assessed by staining, is graded against standard charts.	
NATURE of STUDY	**Nature of study** In this study, 75 patients regarded as having mild to moderate dry eye were assessed for symptoms, MGD, tear quality, meniscus height, blink quality, TBUT F and BR staining, phenol red test and Schirmer. 70.7% female. 61% using ATS 21.9% met European Criteria for moderate to severe dry eye. About 30% were CL wearers.	Nichols et al 2004
CONDUCT of TEST	**Fluorescein instillation:** Fluorescein strip wetted with buffered saline. Drop instilled on inferior palpebral conjunctiva. Blink several times. **Rose Bengal Staining:** A Rosets™ Rose Bengal Ophthalmic Strip is wetted with sterile buffered saline and instilled on the inferior bulbar conjunctiva. ("care taken to instill adequate dye") **STAINING:** 5 corneal regions and 4 conjunctival regions as described in the CLEK study (Barr et al. 1999). The staining scale was 0-4, with 0.5 unit steps in each of the 5 corneal regions. Photos were used as examples of severity. The "total score" could either be summed, or averaged.	Nichols et al 2004 Barr et al 1999 [CLEK study]

OD



OS



C I N T S = Central Inferior Nasal Temporal Superior
0–4 scale in 0.5 unit steps

	circle	location	Check appropriate box				
OD	Location	Cornea/Conj.	Punctate	FB	Coalesced	Full-Thickness	Other
Stain 1	C I N T S						
Stain 2	C I N T S						
Stain 3	C I N T S						
Stain 4	C I N T S						
Stain 5	C I N T S						
Stain 6	C I N T S						
Stain 7	C I N T S						
Stain 8	C I N T S						
Stain 9	C I N T S						

continued

APPENDIX 5 *continued*

<table>
<tr><td>Web Video</td><td>Not available.</td><td></td></tr>
<tr><td>Materials</td><td>• Barnes-Hind Ful-Glo® Fluorescein Sodium Ophthalmic strip
• Rosets™ Rose Bengal Ophthalmic Strip (Chauvin Pharmaceuticals)
• Source of non-preserved buffered saline.</td><td></td></tr>
<tr><td>Standardization</td><td>Nil additional</td><td></td></tr>
<tr><td>Repeatability</td><td>Intra-observer agreement.

Corneal and Conjunctival Staining
Sum of all regions:
Fluorescein stain: The weighted κ was:
0.69 (95% CI = 0.35, 0.81) and the intraclass correlation coefficient was
0.76 (95% CI = 0.58, 0.87).
Bengal rose stain: The weighted κ was:
0.33 (95% CI = 0.45, 0.93) and the intraclass correlation coefficient was
0.40 (95% CI = 0.09, 0.64).

Note that agreement was better for fluorescein than for bengal rose, perhaps because the bengal rose strip gives weaker staining than the fluorescein strip.

Note too, that agreement was less good for individual zones assessed independently as follows:

Unweighted κ for presence versus absence of F and BR staining.
(κ values; [% agreement])

<table><tr><th>Zone</th><th>Cornea Fluor</th><th>Cornea Bengal R</th><th>Conj Fluor</th><th>Conj Bengal R</th></tr><tr><td>Inf</td><td>0.18 (58.7)</td><td>0.02 (81.3)</td><td>0.25 (70.7)</td><td>0.14 (60.0)</td></tr><tr><td>Nas</td><td>0.23 (70.7)</td><td>–0.02(94.7)</td><td>0.14 (56.0)</td><td>0.09 (65.3)</td></tr><tr><td>Temp</td><td>0.47 (82.7)</td><td>0.49 (97.3)</td><td>0.10 (54.7)</td><td>0.46 (92.0)</td></tr><tr><td>Sup</td><td>0.28 (82.7)</td><td>N/A</td><td>0.31 (90.7)</td><td>N/A</td></tr><tr><td>Centr</td><td>0.29 (81.3)</td><td>N/A</td><td></td><td></td></tr></table>N/A Not available because no stain
K values: 0–0.2 slight agreement; 0.21–0.40 fair agreement;
0.41–0.60 moderate agreement; 0.61–<1.0 excellent; 1.0 =perfect agreement

Note, even in region of most frequent corneal staining, κ = 0.21:
It was concluded that perhaps zone scores varied between visits but the total sum of scores was more constant.</td><td>Nichols et al 2004</td></tr>
<tr><td>Test problems</td><td>About 30% were CL wearers. They do not appear to have been analyzed separately.
Only a single observer was involved in the repeatability measurements.
Did patients stop ATS drops before assessment?</td><td></td></tr>
<tr><td>Glossary</td><td>CLEK = Collaborative Longitudinal Evaluation of Keratoconus</td><td></td></tr>
</table>

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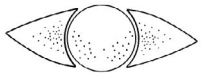
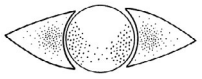
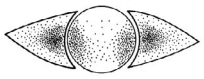
Nichols KK, Mitchell GL, Zadnik K. The repeatability of clinical measurements of dry eye. *Cornea* 2004;23(3):272-85

APPENDIX 6

DEWS	**DRY EYE: DIAGNOSTIC TEST TEMPLATE**	
RAPPORTEUR	A.J.Bron	21st Oct 04
TEST	**GRADING STAINING: Oxford Schema**	
TO DIAGNOSE	The scheme is used to estimate surface damage in dry eye.	REFERENCES
VERSION of TEST	[V 1]	
DESCRIPTION	Surface damage to the exposed eye, assessed by staining, is graded against standard charts.	
CONDUCT of TEST	**Grading Schema:** Staining is represented by punctate dots on a series of panels (A-E). Staining ranges from 0-5 for each panel and 0-15 for the total exposed inter-palpebral conjunctiva and cornea. The dots are ordered on a log scale	Bron Evans Smith 2003

PANEL	Grade	Criteria
A 	0	Equal to or less than panel A
B 	I	Equal to or less than panel B, greater than A
C 	II	Equal to or less than panel C, greater than B
D 	III	Equal to or less than panel D, greater than C
E 	IV	Equal to or less than panel E, greater than D
>E	V	Greater than panel E

Conduct of Test:

- Dye is instilled.
- Slit-lamp is set (eg, 16 magnification with x10 oculars with Haag-Streit).
- Cornea: The upper eyelid is lifted slightly to grade the whole corneal surface,
- Conjunctiva: To grade the temporal zone, the subject looks nasally; to grade the nasal zone the subject looks temporally.
- (The upper and lower conjunctiva can also be graded).

Selection of dyes:

A list dyes and filters can be found in the original paper.
With fluorescein, staining must be graded as quickly as possible after instillation, since the dye then diffuses rapidly into the tissue and its high luminosity blurring the stain margin. Staining after rose bengal or lissamine green, persists at high contrast and may therefore be observed for a considerable period. This is convenient for both grading and photography.

Fluorescein sodium

1. Quantified drop instillation

eg 2 µl of 2% sterile fluorescein instilled into each conjunctival sac with a micro-pipette (using a sterile tip). In very dry eye, larger volumes risk the possibility of inadequate dilution into the fluorescent range.

2. Unquantified instillation — impregnated paper strips

This is a convenient approach in the clinic using the following method of application:

- A single drop of unit dose saline is instilled onto a fluorescein-impregnated strip.
- When the drop has saturated the impregnated tip, the excess is shaken into a waste bin with a sharp flick.
- The right lower lid is then pulled down and the strip is tapped onto the lower tarsal conjunctiva. A similar procedure is carried out on the left.

If too large a volume is delivered then the concentration in the tear film will be too high, and the tear film and staining pattern will be non-fluorescent.

continued

3.Timing
The fluorescein break-up time (FBUT) is usually performed prior to grading staining. Since fluorescein diffuses rapidly into tissues, punctate staining blurs after a short period. It is therefore essential to assess staining rapidly, in sequence, in the right and then the left eye, so that the staining patterns observed are equally crisp.
If it is intended to photograph the staining pattern for grading, then photography should follow immediately after each instillation.

Exciter and Barrier Filters
The absorption peak of fluorescein sodium occurs between 465 - 490 nm and the emission peak between 520 - 530 nm. A suggested filter pair for detection of fluorescein staining is a yellow, Kodak Wratten 12 barrier filter (transmitting above 495 nm) or an orange Wratten 15 filter (transmitting above 510 nm) in combination with a blue Wratten 47 or 47A exciter filter. The 47A shows greater transmittance than the Wratten 47 over the absorption range. The 'cobalt' filter of many slit-lamps is suitable to use with a Wratten 12 or 15 barrier.

Where more light is required for photographic purposes, narrow band-pass, interference filters can be used.

The use of both exciter and barrier filters allows both the cornea and conjunctiva to be assessed using a single stain. This is a major advantage in clinical trials where it is otherwise customary to employ fluorescein to grade corneal staining and rose bengal or lissamine green to grade conjunctival staining.

Disadvantages of Fluorescein Staining
Blurred pattern if reading is delayed. Delay in photographing fluorescein staining results in blurred images of the staining pattern.

Rose Bengal
The intensity of rose bengal staining is dose dependent. If drop size or concentration is reduced to minimize stinging, the amount of staining is also reduced. Use of impregnated strips will give weaker staining than use of a full drop of 1% solution. Best results are achieved with, eg. 25 µl 1%, instilled into the conjunctival sac. Because rose bengal stings, instillation is best preceded by a topical anesthetic.

Instillation Technique
1) eg, a drop of Proxymetacaine is instilled into the conjunctival sac followed, after recovery, by;
2) A drop of rose bengal 1.0%. This is instilled onto the upper bulbar conjunctiva with the upper lid retracted and the patient looking down.
3) Since both anaesthetic and drop may stimulate reflex tearing, the test should follow measurement of the FBUT and of the Schirmer test. (Conjunctival staining due to insertion of the Schirmer paper can usually be distinguished from that due to dry eye disease).

Both eyes may be stained prior to grading, since there is no risk of the staining pattern in the first eye being obscured by the time the second eye is graded.
The cited paper gives advice about avoidance of overspill.

Visibility
Rose bengal staining on the conjunctiva shows up well against the sclera and may be enhanced using a red-free (green) light source. Corneal staining may show up well against a blue iris, but is difficult to see against a dark brown iris.

Phototoxicity
Photo-activation of rose bengal by sunlight increases post-instillation symptoms, especially in severe dry eye with heavy staining. This post-instillation pain can be minimized by liberal irrigation with normal saline at the end of the test.

Lissamine green stains the eye in a similar manner to rose bengal but is as well tolerated as fluorescein. Visibility and dose-dependency are the same as rose bengal and staining is persistant so that photography need not be performed immediately after instillation.
Lissamine green is available as impregnated strips or may be ordered as a pre-prepared solution. A 25 µl 1% drop will give more intense staining. Because the drop is well tolerated, no anaesthetic is required.

continued

APPENDIX 6 *continued*

<table>
<tr><td>CONDUCT of TESTS</td><td>Visibility
As with rose bengal, lissamine green staining is easily visible on the conjunctiva. On the cornea, staining is seen well against a light blue iris background but is poorly visible against a dark brown iris background. For both rose bengal and lissamine green, because the dyes are poorly seen within the tear film, the dye in the tear film does not obscure the staining pattern. Also, since both dyes do not diffuse into the substantia propria of the conjunctiva, the staining pattern is retained for longer.

Visibility of staining may be enhanced using a white light source and a red barrier filter, to give a black pattern on a red ground. A suitable filter is a Hoya 25A, or a Kodak Wratten 92.</td><td></td></tr>
<tr><td>Web Video</td><td>Not available</td><td></td></tr>
<tr><td>Materials</td><td>Oxford grading panel; Slit-lamp; Selected dye.</td><td></td></tr>
<tr><td>Standardization</td><td>See above.</td><td></td></tr>
<tr><td>Repeatability</td><td>A small intra-inter observer study was carried out in 1986 and was presented but not published:

Intra-observer study: This study asked two trained ophthalmologists to grade a series of standard slides, showing corneal and conjunctival fluorescein staining, on 2 separate occasions. [note: -this study is only relevant to grading photographic records not patients.]

Intra-observer κ for grading photographs of staining, using the Oxford scheme. Two observers.
<table><tr><th></th><th>Cornea</th><th>Conjunctiva</th></tr><tr><td>Observer 1</td><td>0.86</td><td>0.69</td></tr><tr><td>Observer 2</td><td>0.65</td><td>0.83</td></tr></table>
Note that values are in the good to excellent range.

Inter-observer study: In this study, the same 2 observers graded fluorescein staining (blue exciter; yellow filter) in 13 dry eye patients at an interval within 2-3 weeks.

Inter-observer κ for grading patients with dry eye, using the Oxford scheme. Two observers. Fluorescein; bengal rose
<table><tr><th>Observer 1 v 2</th><th>Cornea</th><th>Conjunctiva</th></tr><tr><td>Fluorescein</td><td>0.88</td><td>0.48</td></tr><tr><td>Bengal rose</td><td>0.87</td><td>0.54</td></tr></table>
It is of interest that observations are in the excellent category for cornea, with either stain and in the fair category for conjunctiva.</td><td>Hardman Lea et al 1986 AER abstract.</td></tr>
<tr><td>Test problems</td><td>The test depends on pattern recognition applicable to dry eye states.</td><td></td></tr>
<tr><td>Test solutions</td><td>More general use to assess all forms of ocular surface staining can be achieved by scoring staining in multiple segments of the ocular surface while retaining a full number density range of dots</td><td></td></tr>
</table>

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APPENDIX 7

DEWS	DRY EYE: DIAGNOSTIC TEST TEMPLATE	
RAPPORTEUR	Mark B. Abelson and George W. Ousler III	5th Nov 2004
Reviewers	–J Paugh	27th Dec 2007
TEST	**Tear Film Break-Up Time (TFBUT)** also: BUT (Break-up Time) and FBUT (Fluorescein Break-Up Time)	
TO DIAGNOSE	Tear Film Stability	
VERSION	Version I	
DESCRIPTION	The tear film break-up time is defined as the interval between the last complete blink and the first appearance of a dry spot, or disruption in the tear film.	Lemp 1970 Lemp 1995
STUDY	100 subjects with normal ocular health and 100 patients with 'a history of dry eye'. 5 µl of 2% fluorescein were instilled. Average of 3 readings.	Abelson et al 2002
CONDUCT of TEST [V1]	Standardization of the volume instilled is important. Johnson and Murphy 2005 found that increasing the volume of fluorescein instilled from 1–2.7 µl, increased the TFBUT, but that increasing to 7.4 µl was not associated with further change. 1. Instill 1 to 5 micro-liters of non-preserved, 2% sodium fluorescein onto the bulbar conjunctiva without inducing reflex tearing by using a micro-pipette or D.E.T. strip; 2. The patient is instructed to blink naturally, without squeezing, several times to distribute the fluorescein 3. Within 10 - 30 seconds of the fluorescein instillation, the patient is asked to stare straight ahead without blinking, until told otherwise; 4. Set slit-lamp magnification at 10X, keep the background illumination intensity constant (cobalt blue light) and use a Wratten 12 yellow filter to enhance observation of the tear film over the entire cornea; 5. Use stopwatch to record time between last complete blink and first appearance of growing micelle; 6. Once TFBUT is observed, instruct patient to blink freely. Various authors advocate the use of a yellow barrier filter (Kodak Wratten 12) to enhance the visibility of the break in the fluorescent tear film. (Eliason and Maurice 1990; Cho and Brown 1993; Nichols et al. 2003; Bron et al 2003. Johnson et al 2005).	Johnson and Murphy 2005
CONDUCT of TEST [V2]	2.5 µl 1.0% fluorescein	Vitale et al 1994
Results of study	The mean TFBUT for normal subjects was 7.1 s (range 4.7 to 11.4 s) and for dry eye patients 2.2 s (range (0.9 to 5.2 s). On the basis of this, a cut-off for dry eye diagnosis of ≤ 5 s was recommended.	Abelson et al 2002
Video	*Slit-lamp, on-line video camera may be used to capture TFBUT. Video capture with an on-screen timer allows for precise measurement of the time between the last complete blink and the appearance of the first, growing micelle. This also allows masking for clinical trials purposes	Welch et al 2003
Web video	Not available	
Materials	• Non-preserved, 2% sodium fluorescein; • Micro-pipette; • Or D.E.T. strip. • Slit-lamp • Timer • Kodak Wratten filter 12. See variations, below.	
Variations of technique	Historically, the technique for evaluating TFBUT has lacked consistency. Large and varying amounts of sodium fluorescein (up to 50 µl) were used, times were determined by counting aloud and using less sophisticated instrumentation. Such techniques yield varying results.	
Standardization	Time of day [√] Temperature [√] Humidity [√] Air speed [√] Illumination [√] • Patient instruction; • Slit-lamp magnification; • Barrier filter.	

continued

APPENDIX 7 *continued*		
Diagnostic value	This version (micro-quantities of fluorescein): TFBUT ≤ 5 seconds = dry eye; TFBUT > 5 seconds = normal. Other version (larger quantities of fluorescein): TFBUT ≤ 10 seconds = dry eye; TFBUT > 10 seconds = normal.	Lemp 1995 Abelson et al 2002
Sensitivity	(**true positives**) [72.2%] 184/255 patients (cut off ≤ 10 sec)	Vitale et al 1994
Specificity	(**100 – false positives**) [61.6%] 69/112 controls	
Test problems	Instillation of fluorescein must be done carefully so that reflex tearing is not induced. Alterations in tear volume may artificially lengthen TFBUT. Proper patient instruction is critical. If patients are not told to blink freely after TFBUT occurs, reflex tearing may occur and skew subsequent measurements. Large, uncontrolled volumes of fluorescein may also artificially lengthen TFBUT. In the reported study, the age and sex of subjects is not stated and the criteria for dry eye diagnosis are not provided and no sensitivity or specificity calculations were made for the selected cutoff value. However, there was little overlap between the normal and abnormal distribution curves.	 Abelson et al 2002
Glossary	TFBUT = Tear film break-up time: BUT = Break-Up Time) and FBUT = Fluorescein Break-Up Time.	

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APPENDIX 8

DEWS	**DRY EYE: DIAGNOSTIC TEST TEMPLATE**	
RAPPORTEUR	A.J.Bron	19th Oct 2004
TEST	**Schirmer-1 Test — without anesthesia**	
TO DIAGNOSE	Dry Eye	REFERENCES
VERSION	[V1]	
DESCRIPTION	An estimation of tear flow stimulated reflexly by insertion of a filter paper into the conjunctival sac.	
NATURE of STUDY	Diagnostic value of the Schirmer 1 test, Rose bengal staining and a test of lysozyme tear level in sicca syndrome. **Normal** controls: 550 Age 20-74 years M=F in each 5 y band **Sicca** syndrome: 43 F32; M11	
CONDUCT of TEST	**Schirmer-1 test:** The unanesthetized eye Schirmer paper strips Schirmer strips inserted over the lower lid margin, midway between the middle and outer third (assumed). Closed eye (assumed). Read at 5 minutes [No further details]	van Bijsterveld 1969
RESULTS of STUDY	**Schirmer-1:** With a cut of ≤ 5.5 mm the probability of misclassification of patients was 15% and of controls was 17%. No significant differences between men and women at each 5 year age band, but Schirmer value fell with age. Note 107 controls had wetting > 30 mm	
Video need	Not available	
Materials	• Schirmer Papers (5x35mm Whatman No 1)	
Standardization	Time of day [√] Temperature [√] Humidity [√] Air speed [√] Illumination [√]. Assumed to influence.	
Variations of technique	• Calibrated and dyed papers (Eagle Vision - blue) • Paper housed in impervious wrap, to reduce evaporation.	Esquivel and Holly
Sensitivity	**Differentiating 'sicca' from normals:** **(true positives) [85%] ≤ 5.5 mm cut off**	van Bijsterveld 1969
Specificity	**(100 – false positives) [83%] ≤ 5.5 mm cut off**	van Bijsterveld 1969
Test problems	Full details of Schirmer not stated in this paper. Two eye data was pooled for analysis, for all measures (ie. Including rose bengal and lysozyme	
Glossary	'sicca' = keratoconjunctivitis sicca = dry eye. In this study it probably equates with aqueous-deficient dry eye.	

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APPENDIX 9		
DEWS	**DRY EYE: DIAGNOSTIC TEST TEMPLATE**	
RAPPORTEUR	Michael A. Lemp	16th Oct 2004; 15th March 2006
TEST	**Tear Osmolarity**	
TO DIAGNOSE	Global test for dry eye	Sullivan 2004
VERSION of TEST	OcuSense Volume Independent Tear Osmometer	
DESCRIPTION	This "lab-on-a-chip" test uses a combination of impedance information with sophisticated mathematics to derive tear film osmolarity. A small nanoliter tear sample is obtained with a standard micropipette and is then automatically transferred to a chip surface. A precise readout is obtained in seconds after the transfer.	
CONDUCT of TEST	1. Snap microchip in place 2. Touch lower lid with microcapillary 3. Let capillary action draw a few nL 4. Place capillary in machine 5. Read osmolarity	
Web video	Available:[No]	
Materials	• 1-lambda microcapillary • microchip • Both available from OcuSense	
Standardization	Time of day [√] Temperature [√] Humidity [√] Air speed [√] Illumination [√] Assumed to influence Other: [Avoid reflex tearing] White et. al. Showed that use of a slit lamp has upwards of a 7 mOsm/kg effect on the value of osmolality due to the induction of reflex tearing. Overstimulation during collection is discouraged. Reflex tears have far lower osmolality (≈ 5%, Nelson, 1986) than basal tears.	White et al 1993 Nelson et al 1986
Repeatability	Intra-observer agreement. [ ] Inter-observer agreement. [< 2.6% 1st prototype]	Sullivan B 2004
Sensitivity	(true positives) [projected 94%] ≥ 318 mOsm: –provisional	Sullivan B 2004
Specificity	(100 – false positives) [projected 84%]	Sullivan B 2004
Test problems	Limited availability	
Test solutions	Commercial development	
FORWARD LOOK	This is a high throughput test that can be performed by a technician, and currently carries a miscellaneous CPT.	

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APPENDIX 10

DEWS	DRY EYE: DIAGNOSTIC TEST TEMPLATE	
RAPPORTEUR	Mark Willcox	10th Jan 2006
TEST	**Tear meniscus radius, height and cross sectional area**	
TO DIAGNOSE	Aqueous tear deficiency (ATD).	REFERENCES
VERSION	[V 1] **Meniscometry**	Yokoi Komuro 2004
DESCRIPTION	A rotatable projection system with a target comprising black and white stripes is projected onto the lower central tear film meniscus. Images are recorded and transferred to computer in order to calculate radius of curvature	
CONDUCT of TEST	1. The subject is seated at a slit lamp 2. A rotatable projection system with a target comprising a series of black and white stripes (4 black and 5 white; each 4mm wide), is introduced coaxially using a half-silvered mirror 3. Images of the tear meniscus (of either or both eyes) are recorded with a digital video recorder 4. Images are transferred to a computer and image analysis software used to calculate the radius of curvature of the meniscus by applying the concave mirror formula	
Web Video	Not available	
Materials:	• Slit lamp • Rotatable projection system (see above) with half silvered mirror • Digital video recorder plus TV monitor • Computer plus software • Colour printer	Oguz et al 2000
Variations of technique	Several alternative methods have been published including: 1. Use of variable beam height on a slit lamp 2. Measurement and grading of meniscus integrity using slit lamp 3. Using a video slit lamp biomicroscope but no projected stripes 4. Measurement after instillation of fluorescein	Nichols et al 2004a Cermak et al 2003 Glasson et al 2003 Farrell et al 2003 Oguz et al 2000
Standardisation	Assumed to be influenced by: Time of day [√] Temperature [√] Humidity [√] Air speed [√] Illumination [√]	
Repeatability	Intra-observer agreement. [Not recorded for V1 – but poor in Nichols et al system]	
Sensitivity	Tear meniscus height: cut off of: < 0.18 mm (true positives) Farrell et al's technique = [72.8%]	Farrell et al 2003
Specificity	(100 – false positives) Farrell's technique = [66.6%]	
Sensitivity	Tear Meniscus Height: Small vol. fluorescein: cut off < 0.35mm (true positives) Mainstone et al = [93.3%]	Mainstone et al 1996
Specificity	(100 – false positives) Mainstone et al = [66.7%]	
Other Stats	For V1 – significantly lower meniscus height in dry eye subjects. Plugging puncta significantly increased meniscus height. Significant correlation between meniscus height and Schirmer test Cermak et al – significantly lower meniscus height in androgen insensitive female subjects who demonstrated dry eyes Farrell et al – significant decrease in dry eye subjects compared with controls; significant increase in dry eye subjects with puncta occluded Correlations noted between meniscus curvature and meniscus height in presence or absence of fluorescein Tear meniscus height and area reduced in subjects intolerant to contact lens wear compared with tolerant subjects Nichols et al (2004b) demonstrated lack of association between tear meniscus height and symptoms of dry eye.	Yokoi and Komuro 2004 Cermak et al 2003 Farrell et al 2003 Oguz et al 2000 Glasson et al 2003 Nichols et al 2004b
Test problems	Positioning of subject etc and use of specialized equipment	
Forward Look	To adapt the V1 method for general use.	

continued

APPENDIX 10 *continued*

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APPENDIX 11

DEWS	DRY EYE: DIAGNOSTIC TEST TEMPLATE	
RAPPORTEUR	Eiki Goto, MD	15th Mar 2006
TEST	**Tear film lipid layer interferometry**	
TO DIAGNOSE	Aqueous tear deficient dry eye (ATD) or precorneal lipid tear deficiency.	REFERENCES
VERSION	[V6]	Goto et al 2003
DESCRIPTION	Superficial tear lipid layer is observed with tear interference camera. Interference images are graded on dry eye severity or analyzed to quantify lipid layer thickness.	Korb and Greiner 1994; King-Smith et al 1999; Yokoi et al 1996; Mathers et al 1997; Goto et al 2003
CONDUCT of TEST	1. The subject is seated comfortably at the tear interference camera and the head positioned on the chin rest. 2. With the eyes in normal blinking interference images are monitored. 3. After a few seconds of blinking, when the interference image becomes stable, the image is captured. 4. Lipid layer thickness is estimated using a color comparison table (Korb and Greiner). 5. Interference images are semi-quantitatively graded on the pattern and color. (Yokoi et al) 6. In a kinetic analysis, interference images are recorded on a video over several natural blink intervals for 30 seconds. In a representative blink interval, lipid spread time from eye opening to the cessation of lipid movement is measured. (Goto and Tseng) 7. When image analysis is needed, the captured, still, interference image is analyzed by its colour profile. Lipid layer thickness is quantified with the color chart system. (Goto et al)	Doane 1989; Korb and Greiner 1994; Yokoi et al 1996; Goto and Tseng 2003 Goto et al 2003 Korb et al 2005
Web Video	Not available	
Materials	• Tear interference camera (DR-1, Kowa, Nagoya, Japan), Dr. Korb's camera, Dr. Doane's camera or Tearscope (Keeler, Windsor) • Digital printer • Hopefully PC for image capturing	Yokoi et al 1996 Goto and Tseng 2003
Standardization	Time of day [√] Temperature [√] Humidity [√] Air speed [√] Illumination [√] Other: [blinking √]. Assumed to influence	
Variations of technique	V1, Tear lipid layer interference images were observed using devices such as Tearscope. V2, Lipid layer thickness was estimated using color comparison method. V3, Images were captured using modified specular microscope and graded on dry eye severity in Sjogren syndrome. V4, Interference camera was sophisticated (DR-1, Kowa, Japan) and images were graded on dry eye severity. V5, Kinetic analysis of interference images using DR-1 to measure lipid spread time. V6, Precorneal lipid layer thickness was quantified using colorimetric system in DR-1. V7, Lipid layer thickness topography was processed. * Tear interference patterns on contact lens are also evaluated by Guillon or Maruyama.	Guillon 1992 Korb and Greiner 1994 Danjo and Hamano 1995 Yokoi et al 1996 Tiffany et al 2001 Goto and Tseng 2003 Goto et al 2003 Goto et al 2004 Maruyama et al 2004
Diagnostic value	See references 4 and 5.	Yokoi et al 1996 Yokoi et al 1999
Repeatability	Intra-observer agreement. [+], V4 on grading and V5 on grading and Kinetic analysis Inter-observer agreement. [–]	Yokoi et al 1996; Yokoi et al 1999; Goto and Tseng 2003; Goto and Tseng 2003

continued

APPENDIX 11 *continued*		
Test problems	a. Colour intensity of interference images are influenced by the refractive indices of tear lipid and aqueous layers and specular angle. b. Interference images are influenced by how to blink, thus to record the non-invasive status of the lipid layer, it is important for the subject to blink naturally. c. Lipid quality could not be indicated by interferometry. d. Amount of meibum secretion observed at lid margin does not always correlate with the precorneal lipid layer thickness (a phenomenon, not a test problem)	Goto et al 2003 King-Smith et al 1999 Tiffany 1986
Test solutions	a. Image analysis for lipid thickness quantification need to be developed more.	
FORWARD LOOK	a. Identify cut-off for MGD, and ATD diagnosis. b. Incorporate MGD diagnosis into diagnosis of evaporative dry eye or precorneal lipid deficiency. c. Image analysis on raw interference image and quantification of lipid layer thickness in a mapping form. Clinically useful index from mapping for comparison and stats.	
Glossary	ATD = Aqueous tear deficient dry eye	

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APPENDIX 12

DEW	DRY EYE: DIAGNOSTIC TEST TEMPLATE	
RAPPORTEUR	Murat Dogru	24th Oct 2004
TEST	**Tear Stability Analyses System (TSAS)**	
TO DIAGNOSE	Test used to diagnose –Tear Instability Refs:	Kojima 2004 Goto 2004a,b
VERSION	[TMS-2N]	Kojima 2004
DESCRIPTION	Noninvasive and objective test for tear film stability analysis	
Study	To compare the sensitivity and specificity of TSAS with the BUT (based on slit-lamp examination and use of fluorescein), 48 volunteers without any eye disease, surgery or drug use within 1 year of study were recruited. See below.	Goto 2004a
CONDUCT of TEST	Subject seated in front of TMS-2N corneal topography unit. Subject asked not to blink for 10 seconds with test initiation Device automatically captures corneal topograms each second for 11 consecutive seconds, displayed as time plot curves of SRI, SAI, BUT area	
Results of Study	See study, above. 42.5% (34 eyes) of the 80 eyes of the volunteers studied had a normal BUT and 57.5% had an abnormal BUT. On the basis of the subjects' dry eye symptoms such as FBS, soreness, dryness etc, the sensitivity and specificity of the BUT were 75% and 60% respectively. Among 34 eyes with a normal BUT, 11 (32.35%) were found to have an abnormal TMS BUT. Of these eyes, 9 (81.8%) were from 6 subjects who had dry eye symptoms in their questionnaires. On the basis of symptomatology, the sensitivity and specificity of TMS BUT was 97.5 and 62.5% respectively. The difference of sensitivity between SLE BUT and TMS BUT was significant; however, the difference in specificity was not.	
Web Video	Not available	
Materials	TMS-2N corneal topography device TSAS software(Tomey Inc)	
Standardization	Time of day [√] Temperature [√] Humidity [√] Air speed [√] Illumination [√] . Assumed to influence.	
Sensitivity	(true positives) [97.5%]	Goto 2004a
Specificity	(100 – false positives) [62.5 %]	
Test problems	Although the test appears to be a promising, non-invasive method to test tear stability, it is not known whether the test is evaluating tear stability due to lipid layer or overall tear film changes. Only one study compares the test with the invasive fluorescein aided BUT measurement. Normal values of this test and age-specific cut off values on a large set of subjects not yet established. Comparative studies with other invasive and non-invasive tests of tear stability do not exist as yet. Needs a corneal topography device and the software which makes it expensive compared to fluorescein aided BUT testing.	
Test solutions	The above mentioned studies will prepare this test for general clinical prime time.	
Forward Look	The device is still being furnished with novel parameters such as BUT area. For dynamic analyses of tear functions in dry eye syndromes and ocular surface disorders, I believe that this new system is set to play an important role in the future.	
Glossary	TSAS: Tear Stability Analyses System	

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APPENDIX 13

DEWS	**DRY EYE: DIAGNOSTIC TEST TEMPLATE**	
RAPPORTEUR	John M. Tiffany	12th Nov 2004
TEST	**MEIBOMETRY**	
TO DIAGNOSE	Meibomian Gland Dysfunction — (MGD)	REFERENCES
VERSION of TEST	[V1]	Komuro et al 2002
DESCRIPTION	Lipid on the lower central lid margin is blotted onto a plastic tape and the amount taken up read by optical densitometry. This provides an indirect measure of the steady state level of meibomian lipid.	
CONDUCT of TEST	1. The subject is seated, with the head resting comfortably at the slit-lamp. 2. With the eyes in upgaze, the right lower lid is drawn down lightly without pressure on the tarsal plate. 3. A standard loop of plastic tape, held in an applanation or ultrasonography probe holder, is applied to the central third of the everted lid margin for 3 seconds, at 0 mmHg exerted pressure. 4. The tape is air dried for 3 minutes to allow tear evaporation if necessary. 5. The increase in transparency induced by the lipid blot, is read in the laser meibometer. 6. The Casual Lipid level (expressed as arbitrary optical density units) is calculated as (C-B), where C is the casual reading, B is the reading from the untouched tape (background).	Komuro et al 2002
Video need	Not available.	
Materials	• Plastic tape: 8 mm wide (Courage and Khazaka, Köln) • Tape Holder:(eg. NIDEK ultrasonographic probe holder. • Laser Meibometer. Window size (2.5 x 5.0 mm2)	
Standardization	Time of day [x] The level is highest in the first hour after waking, but thereafter settles to a constant level through most of the day	
Variations of technique	In the original version, [V2] optical density was read using an adaptation of the Courage and Khazaka sebumeter. A point reading was taken at the centre of the blot. Other methods exist in which the blot is scanned and the increase in transparency is integrated over the length of the blot . The spring-clip holding the loop of tape can be mounted with wax, modeling clay or "Blu-Tack" to the end of a thin wooden rod (eg, a bamboo kitchen skewer) held upright by a lump of wax to the ultrasonography mounting-plate; this also exerts zero pressure on the eyelid. After blotting, the loop is opened and attached to a highly-reflective surface (mirror or polished metal) for scanning.	Chew et al 1993a,b Yokoi et al 1999
Test problems	a. In normal subjects the lipid blot is uniform and results can be extrapolated to the total lid length. In MGD, focal gland obstruction may vary along the lid length so that central readings may not truly reflect the overall picture. b. Calibrations and assumptions are required to convert raw densitometry readings into meibomian lipid equivalent values.	
Test solutions	a. Measurement should be made along the whole of the lower lid length in order to reflect variation in MGD. b. If the scanning method is used, either a maximally-wide or a very narrow area across the blot should be integrated, to give either an averaged reading including regions with non-functional glands, or a reading only from a selected area of full blotting.	
Forward Look	a. Develop a system to integrate lipid along full lid length. b. Identify cut-off for MGD diagnosis. c. Incorporate MGD diagnosis into diagnosis of evaporative dry eye.	
Glossary	MGD: Meibomian gland dysfunction	

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APPENDIX 14

DEWS	**DRY EYE: DIAGNOSTIC TEST TEMPLATE**	
RAPPORTEUR	Gary N. Foulks	19th Oct 04
TEST	**MEIBOGRAPHY/MEIBOSCOPY**	REFERENCES
TO DIAGNOSE	Meibomian gland morphology and density and drop out. Diagnosis of Meibomian gland dysfunction (MGD)	Robin et al 1985 Jester et al 1982
VERSION	[V1]	*reference 1 above*
DESCRIPTION	Meiboscopy is the visualization of the meibomian gland by transillumination of the eyelid. Meibography implies photographic documentation	Mathers et al 1994
CONDUCT of TEST	Meiboscopy: The most basic version uses white light from a Finoff transilluminator. This is applied to the cutaneous side of the everted eyelid and allows observation from the conjunctival surface The presence and morphology of the glands can be observed and gland loss, or "drop out" quantified. Meibography is the photographic documentation of the image of the gland under such illumination. Variations on the theme include the use of infrared photography or videophotography.	
Web Video	Not available	
Materials	• Finoff head light, slit lamp biomicroscope • (variation: infrared light source and sensor; videography)	
Variations of technique	1) infrared photography 2) videography Variations in scoring systems.	Pflugfelder 1998 Shimazaki 1998 Yokoi 2007
Standardization	Illumination [√]	
Diagnostic value	This version : [x] Most reliable test in patients with ectodermal dysplasia syndrome Other version: [ ]	Kaercher et al 2004
Other Stats	Greatest value is determining presence or absence of gland. Morphological variations, while interesting, are more difficult to quantify.	
Test problems	The limitation is the subjective nature of the observation.	
Test solutions	An improvement could be standardized photographs as reference.	
Forward Look	Improved photographic documentation.	
Glossary	MGD: Meibomian gland dysfunction	

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APPENDIX 15

DEWS	DRY EYE: DIAGNOSTIC TEST TEMPLATE	
RAPPORTEUR	Kazuo Tsubota	14th Dec 2004
TEST	**Brush Cytology Technique**	
TO DIAGNOSE	A variety of ocular surface diseases	REFERENCES
VERSION	[1]	
DESCRIPTION	Brush cytology is the technique which collects conjunctival epithelial samples from the patient, clinically. This method is different from impression cytology in that brush cytology can obtain basal cells as well as superficial cells.	Tsubota 1990 (a) Tsubota 1990 (b) Tsubota, 1991 Fukagawa 1993 Fujihara 1997 Miyoshi 2001 Takano 2004
CONDUCT of TEST	Brushing cytology of the conjunctiva is a moderately invasive but can provide a valuable snapshot of the surface of the eye to evaluate many conjunctival conditions.	
Video needed	Not available	
Materials	• Small Brush (Teikokuzouki Pty. Ltd., Japan), • Hank's buffered solution, • Millipore filter (Millipore Corp., Bedford, MA)	
Standardization	The strength of the pressure applied to the conjunctiva by brush should be moderate.	
Diagnostic value	This version is useful to evaluate: 1) squamous metaplasia, 2) detecting inflammatory cells, 3) expression of several surface markers on the ocular surface epithelium.	Tsubota 1990 (b)
Test problems	The procedure is slightly invasive to the patient as the cells are detached from the ocular surface	
Test solutions	Use a very soft brush (do not use a rough brush)	
Forward Look	Since more than 100,000 cells are obtained using brush cytology, this is a very good technique to see molecular expression by each cell. Thus this technique, combined with flow cytometry can give us more detailed information about events at the ocular surface at the cellular level.	

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APPENDIX 16

DEWS	**DRY EYE: DIAGNOSTIC TEST TEMPLATE**	
RAPPORTEUR	Christophe Baudouin	7th Nov 2004
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 specially relevant in dry eye, allergy or assessment of antiglaucoma eyedrops	Brignole et al 2000, 2001
CONDUCT of TEST	1. Without or under topical anesthesia 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, X100 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	
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 Please cite statistics indicating the diagnostic value of the test.	Brignole et al 2004
Repeatability	Standardized technique reliable over time and from one laboratory to another	
Test problems	This procedure is highly technical and requires a 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	
Glossary	HLA-DR: Major leukocyte antigen, human histocompatibility complex, class II cell surface receptor	

continued

APPENDIX 16 *continued*

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APPENDIX 17

DEWS	DRY EYE: DIAGNOSTIC TEST TEMPLATE	
RAPPORTEUR	Maurizio Rolando	1st Nov 2004 11th Jan 2006
TEST	**Ferning Test (TFT)**	REFERENCES
TO DIAGNOSE	Quality of tears (electrolyte concentration), KCS, Hyperosmolarity	
VERSION of TEST	[V1] Tear ferning test (tear collection by rod) [V2] Tear collection by glass capillary)	Rolando 1984 Norn 1994
DESCRIPTION	A drop of tears is collected from the lower meniscus and dropped onto a microscope slide and allowed to dry by evaporation. Different forms of branching crystallization patterns can be observed and classified. The tear ferning test permits separation of normal from dry eyes on the basis of the ferning patterns.	Golding et al 1994 Rolando 1986-1988 Pearce, Tomlinson 2000
CONDUCT of TEST	1. The subject is seated, with the head resting comfortably, in a dim light. 2. With the eyes in upgaze, by means of a micropipette, nearly 1 microliter of tears is collected by capillarity from the lacrimal river of the lower meniscus. 3. The fluid is then dropped onto a microscope slide and exposed to evaporation at 20 ±3 C° for 10 minutes 4. The sample is then observed under a microscope at x 100-400 enlargement (better visibility is achieved with phase contrast microscopy) 5. The patterns of crystallization (ferning) are classified in 4 classes: Type 1: uniform large arborization, Type 2: ferning abundant but of smaller size; Type 3: partially present incomplete ferning; Type 4: no ferning. Types 1 & 2 are reported to be normal and Types 3 & 4 reported to be abnormal	Rolando 1984-1986
Web Video	Not available	
Materials	• capillary glass • clean microscope slides [ ] • light microscope (Phase contrast useful but not necessary)	
Standardization	Time of day: [any] Temperature: [20-28°C] Humidity: [high humidity slows down the time of appearance of the ferns] Air speed: [the effect of excessive air speed has not been studied but increasing the evaporation rate could affect the pattern of ferning]. Illumination: [the level of illumination seems irrelevant in the development of ferning patterns once the sample has been collected and dropped] Other: [Avoid excessive light and lid margin contact in order to decrease reflex tearing.]	
Variations of technique	In the original version, [V1] tear collection was acheived by capillary attraction by means of a 0.5 mm rod loop placed in contact with tears pooled in the lower fornix of the cul de sac The second version uses a capillary tube in contact with the fluid of the lower meniscus. This increases reproducibility, with a coefficient of variation of 6.4%.	Norn 1994
Diagnostic value	This version: [ ] Other version: [2] prognostic value 86.6%	Albach et al 1994
Repeatability	Intra-observer agreement. [Intraobserver agreement of 94.50% (kappa = 0.76; CI = 0.67-0.86). -] Inter-observer agreement. [Interobserver agreement 92.10% (kappa = 0.65; CI = 0.56-0.75]	Pensyl and Dillehay 1998
Sensitivity	(true positives) [82.2%] [Cut off: Type III or worse according to the previously reported classification 6-7)	Albach et al 1994
Specificity	(100 – false positives) [92.5%]	Albach et al 1994
Other Stats	94% sensitivity 75% specificity [Cut off: Type III or worse according to the previously reported classification 6-7] 92% sensitivity 83% specificity [Cut off: Type III or worse according to the previously reported classification 6-7]	Norn 1994 Rolando 1986

continued

APPENDIX 17 *continued*		
Test problems	Care should be taken not to elicit reflex tearing during collections Light microscopy is often unavailable in the office. In spite of a good clinical ability of separating normal from dry eyes, the real meaning of the results is not known [Test affected by extreme conditions of temperature and humidity]	
Forward Look	It would be interesting to explore the correlation between the patterns of crystallization (test types I to IV) and the level of tear film osmolarity	
Glossary	TFT: Tear ferning test	

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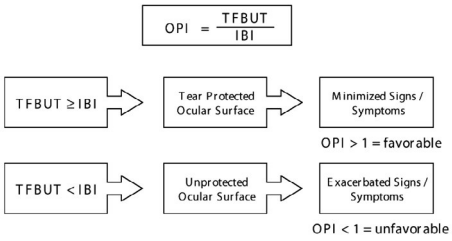
APPENDIX 18

DEWS	DRY EYE: DIAGNOSTIC TEST TEMPLATE	
RAPPORTEUR	Mark B. Abelson and George W. Ousler III	5th Nov 2004
TEST	Ocular Protection Index (OPI)	Ousler et al 2002
TO DIAGNOSE	Ocular Surface Protection Risk of ocular surface damage	
VERSION	[V1]	
DESCRIPTION	The principle of the test is that when the tear film break up time (TFBUT) is shorter than the blink interval (IBI), the eyes are exposed to the risk of focal ocular surface damage. The Ocular Protection Index (OPI) is the ratio of the TFBUT and IBI (TFBUT/IBI). If the OPI score is < 1, then a patient's cornea is at risk of exposure and if the OPI score is ≥ 1, it's not.	Ousler et al 2002
General note	When studying the relationship between TFBUT and the inter-blink interval (IBI = time between complete blinks), it may be suggested that their interaction assists in regulating the integrity of an ocular surface. For example, the ocular surface is protected when the TFBUT either matches or exceeds than the IBI. In contrast, the surface is unprotected surface when the TFBUT is less than the IBI. This relationship can be clinically relevant since repeated, intermittent exposures of a tear film deficient cornea lead to symptoms and signs such as keratitis and redness. An index known as the Ocular Protection Index (OPI) can be used to quantify the interaction between the IBI and TFBUT. The OPI is calculated by dividing TFBUT by the IBI. If the OPI score is < 1, a patient's cornea is at risk for exposure, and if the OPI score is ≥ 1, it's not. This approach to measuring alterations in TFBUT has proven to be useful in assessing factors that cause dry eye and evaluating therapies.	
CONDUCT of TEST	1. Complete a visual count of the number of blinks per minute while your patient reads the ETDRS chart; 2. Calculate IBI = 60 divided by the number of blinks per minute; 3. Measure TFBUT; 4. Divide TFBUT by the IBI to determine OPI score – Ocular Protection Index (OPI)  	Ousler et al 2002
Web Video	Not available	
Materials	Blink Rate Recorder – • ETDRS chart or standard visual task; TFBUT Measurement – • Non-preserved, 2% sodium fluorescein; • Micro-pipette; • *Or* D.E.T. strip.	See TFBUT template for details of TFBUT test
Standardization	Time of day [√] Temperature [√] Humidity [√] Air speed [√] Illumination [√]	
Diagnostic value	OPI Score ≥ 1 = protected ocular surface OPI Score < 1 = unprotected ocular surface	Ousler et al 2002 Abelson et al 2002
Glossary	OPI = Ocular Protection Index: TFBUT =Tear film break-up time: IBI = Inter-blink Interval:	

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APPENDIX 19

DEW	**DRY EYE DIAGNOSTIC TEST TEMPLATE**	
RAPPORTEUR	Alan Tomlinson	10th Jan 2006
TEST	**Fluorophotometry (Fluorimetry) – Tear Flow**	
DIAGNOSES	Changes in tear flow in aqueous tear deficiency (ATD).	REFERENCES
VERSION of TEST	[Version 1] Scanning automated fluorophotometry (Fluorotron Master, Coherent Inc, Palo, Alto, CA)	
DESCRIPTION	To calculate tear flow from measurements of tear volume and turnover.	
CONDUCT of TEST	**Tear Turnover Rate** 1) Subject is seated at the chin rest of the Fluorotron (with the anterior segment adapter fitted). Horizontal and vertical adjustments are made to align the subject's eye in the instrument's optic beam. 2) Three scans are conducted to establish the intrinsic corneal autofluorescence. 3) A 1 µl drop of 2% sodium fluorescein is instilled into the lower fornix with a pipette. 4) Initial scans are taken 1 minute post instillation, then at 2 minute intervals for a further 20 minutes. 5) The intrinsic corneal autofluorescence value is substracted from all values obtained from tear film fluorescence, prior to data analysis. 6) Fluoroscein concentration at each time point is calculated from the Fluorotron scans obtained at all time points beyond 4 minute post instillation, to avoid initial reflex tearing caused by instillation. 7) The decay in fluorescence is calculated from the log of the curve obtained from the formula: $T_0(t_0) = 100\ \frac{[C_t(t_0) - C_t(t_0+1]}{C_t(t_0)}$ (%/min) Where Ct(t) = fluorescein concentration in tear film at time t(min). Assuming a monophasic decay of fluorescence from 5 mins post instillation with a decay time constant β (min^{-1}): $C_t(t) = C_t\ (0).e^{\beta t}$ (ng/ml) the following is obtained: $T_t(t_0) = 100\ (1 - e^{\beta t})$ (%/min) This calculation can be carried out using the software package ANT_SEGMENT tear. **Tear Volume** 1) Subject is seated at the chin rest of the Fluorotron (with the anterior segment adapter fitted). Horizontal and vertical adjustments are made to align the subject's eye in the instrument's optic beam. 2) Three scans are conducted to establish the intrinsic corneal autofluorescence. 3) One µl of 2% sodium fluorescein is instilled into the lower fornix with a pipette. 4) Initial scans are taken 1 minute post instillation, then at 1 minute intervals for a further 4 minutes. 5) The intrinsic corneal autofluorescence value is substracted from all values obtained from tear film fluorescence, prior to data analysis. 6) Fluorescein concentration at each time point is calculated from all the Fluorotron scans obtained. 7) The decay in fluorescence is calculated from the log of the curve obtained from the formula: $T_0(t_0) = 100\ \frac{[C_t(t_0) - C_t(t_0+1]}{C_t(t_0)}$ (%/min) Where $C_t(t)$ = fluorescein concentration in tear film at time t(min). Assuming a monophasic decay of fluorescence from 5 mins post instillation with a decay time constant β (min^{-1}): $C_t(t) = C_t\ (0).e^{\beta t}$ (ng/ml) the following is obtained: $T_t(t_0) = 100\ (1 - e^{\beta t})$ (%/min) This calculation can be carried out using the software package ANT_SEGMENT tear. Tear volume is then calculated from: $V_t = (C_d.C_m^{-1}.k^{-1}–1)\ V_d$ Where C_d = fluorescein concentration in the drop C_m = initial fluoroscein concentration calculated by back extrapolation with the Fluorotron in ng/ml k = correction factor (k = 250) for the limited spatial resolution of the Fluorotron and V_d = drop volume in ml Calculation of tear flow: Tear flow = $\frac{V_t}{T_0(t_0)}$ (µl/min)	Kuppens 1992 Van Best 1995 Van Best 1995 Kuppens 1992 Van Best 1995 Kuppens 1992 Mishima 1965

continued

APPENDIX 19 *continued*

Web Video	Not available	
Materials	Fluorotron Master 2% sodium fluorescein Mimims (Chauvin, UK) Air displacement pipette P2 Pipetman (Gilson, Villiers-le-Bel, France) Disposable sterile tips (Gilson, Villiers-le-Bel, France)	
Variations of technique	Varying concentrations and instillation volumes of fluorescein can be used, eg, 1% and 0.5 -2 µl.	
Standardization	Time of day [X] Temperature [ ] Humidity [ ] Air speed [still] Illumination [low ambient] Other: [Blink is initiated immediately prior to scan to ensure uniform tear thickness]	Pearce et al 2000
Diagnostic value	This version: [ ] Determination of tear flow an indication of aqueous tear deficiency. To obtain estimate of tear drainage from eye. Other version: [ ]	Mathers, Daley 1996 Mathers et al 1996 Gobbels et al 1992
Repeatability	*Intra-observer variation. [Not significant]* *Inter-observer variation. [Not significant]*	Mishima et al 1966 Van Best 1995
Test problems	High cost of basic equipment. Time required for measurement. Indirect (surrogate) measures of tear outflow and volume as it is assumed that fluorescein and aqueous tear are eliminated at the same rate from the eye. Absorption of fluorescein into the ocular tissue may be a factor in dry eye patients and may decrease apparent rate of decay.	
Test solutions	Use of high molecular weight conjugates.	McNamara et al 1998
Forward Look	Production of a cheaper automated scanning fluorophotometer. Development of reduced test incorporating 6 measurements for total of 10 minutes (tear turnover). Combination of tear flow (µl/min) with evaporation rate (µl/min) gives a value of "total tear flow" in the eye and an estimate of total tear production. This allows analysis of the proportion of tears eliminated by evaporation and/or drainage in various forms of dry eye.	Pearce et al 2000 Mathers, Daley 1996 Mathers 2004

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APPENDIX 20

DEW	DRY EYE: DIAGNOSTIC TEST TEMPLATE	
RAPPORTEUR	Stephen Kaye	18th April 2006
TEST	**Tear Function Index (*Liverpool modification*)** Email: TFI@clineng-liverpool-nhs.com	
TO DIAGNOSE	To evaluate the tear dynamics of production and drainage and detect subjects suffering from dry eye	Ono et al 1991 Xu et al 1995(a) Xu et al 1995(b) Kaye et al 2001
VERSION of TEST	The test is a modification of that described by Xu et al. (1995) and depends on using prepared filter paper strips containing fluorescein. The test has been designed to allow direct measurement of the TFI using prepared tear strips.	Kaye et al 2001
DESCRIPTION	TFI is the quotient of the Schirmer test value and the Tear clearance rate (TCR).	
CONDUCT of TEST	A fluorescein-coated tear strip is placed over the lower lid margin at the junction of the middle and lateral third of the lid. 1. The eye is closed and the strip is left in place for 3 minutes 2. On removal, the distance from the strip notch to the wetted dye front is recorded, using the scale provided. 3. The strip is air dried and 4. The intensity of staining is compared with that of the calibrated panel of dilutions, (ranging from 1:1 to 1:128), to determine the TCR. 5. The TFI is defined as the quotient of the Schirmer test and the TCR.	
Web Video	Not available	
Materials	• The standard kit provides a cardboard envelope, containing a docket with 4 see-through pouches. • Each pouch contains 4 sterile, single-use, fluorescein-coated tear-strips together with a calibrated colour scale for reference. • A ruled measurement scale is printed on the envelope, together with • a nomogram and • a set of instructions The kit, containing the prepared strips, together with instructions and calibrated measuring scale and colour scale are provided by the Dept. Clinical Engineering of the Royal Liverpool University Hospital, Prescot Street Liverpool L7 8XP. For further information: Email: TFI@clineng-liverpool-nhs.com	
Variations of technique	TFI as described by Xu et al (1995)	
Standardization	The procedure is standardised. Strips are calibrated for use in each pack.	
Diagnostic value	Identification of subjects suffering from aqueous tear deficiency, for instance in Sjögrens syndrome.	
Sensitivity	A TFI of less than 40 is 100% sensitive for patients with SS dry eye	Kaye et al 2001
Specificity	Patients with Sjögren's syndrome have a TFI upper 95% confidence interval of 15 (12 if anaesthetic has been used)	Kaye et al 2001
Other Stats	Less inter-ocular difference and less variability than the original method	Kaye et al 2001
Test problems	As with the Schirmer's test, it is uncomfortable. Also, staining of the ocular surface at the sites of strip contact with the conjunctiva occur after using fluorescein or Rose Bengal.	
FORWARD LOOK	Performing the TFI using prepared filter paper strips with the matched colour dilution is very sensitive for detecting patients with SS dry eye. The test can be used by non-ophthalmically trained personel. Subjects with a TFI of less than 40 can then be referred for an ophthalmic assessment.	
Glossary	TFI: Tear function index	

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