Protocol OSL-2016-1007

Comparative evaluation of clinical methods of tear film stability assessment: a randomised crossover study

Protocol Date:	January 27, 2016
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Sponsor:	None
Study Site:	Ocular Surface Laboratory Department of Ophthalmology New Zealand National Eye Centre The University of Auckland Private Bag 92019 Auckland, New Zealand
Principal Investigator:	Jennifer P. Craig, PhD, MCOptom (TPA NZ) Associate Professor Email: jp.craig@auckland.ac.nz Phone: +64 9 923 8173

SIGNATURE OF AGREEMENT FOR PROTOCOL OSL-2016-1007

I have read this protocol and agree to conduct the study as outlined herein in accordance with current Good Clinical Practice (GCP) and ICH Guidelines and in compliance with the obligations and requirements of clinical investigators set forth by local government agencies.

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Principal Investigator Name Date

_____

Principal Investigator Signature

24 **Table of Contents** **Page**
25

Protocol Synopsis	3
1. Introduction and Rationale	4
2. Study Objectives	4
3. Study Design	4
4. Participant Enrolment	5
4.1. Power Calculations	5
4.2. Eligibility Criteria	5
4.3. Informed Consent	5
5. Study Visit Description	5
6. Statistical Analysis Plan	6
6.1. Normality Assessment	6
6.2 Primary Analysis	6
6.3. Secondary Analysis	6
7. Ethical and Regulatory Compliance	6
7.1. Good Clinical Practice Statement	6
7.2. Informed Consent	7
7.3. Subject Confidentiality and Data Protection	7
7.4. Institutional Review Board	7
8. Protocol Amendments	7
9. Study Documentation	8
9.1. Retention of records	8
10. References	9
Appendix A: Study Design	10
Appendix B: Clinical Measurements	11

26

Protocol Synopsis

Title	Comparative evaluation of clinical methods of tear film stability assessment: a randomised crossover study
Site location	Ocular Surface Laboratory Department of Ophthalmology New Zealand National Eye Centre The University of Auckland Private Bag 92019 Auckland, New Zealand
Principal investigator	Jennifer P. Craig, PhD, MCOptom (TPA NZ)
Study duration	12 months
Estimated study completion date	December 2016
Study design	Prospective, investigator-masked, randomised crossover study
Population	A minimum of 62 participants will be recruited into two equal-sized age, gender, and ethnicity-matched groups, with and without symptomatic dry eye (Ocular Surface Disease Index ≥13).
Intervention	The tear film breakup time of participants will be assessed in triplicate, with and without sodium fluorescein instillation (TBUT and NIKBUT), in a randomised order, by two independent observers to ensure investigator masking. A 30-minute interval between measurements will ensure for subsidence of any reflex tearing. In the intervention arm, sodium fluorescein will be applied to the bulbar conjunctiva from a fluorescein impregnated strip (Haag-Streit) wetted with 1 drop (10µl) of saline and shaken to remove excess fluid. Following fluorescein instillation, participants will be instructed to blink naturally over a 1 minute period to facilitate even distribution over the ocular surface. Tear film breakup time will then be assessed in triplicate under blue light with a Wratten yellow filter. In the control arm, tear film breakup time will be measured using an automated non-invasive measurement technique (Oculus Keratograph® 5M, Germany) without sodium fluorescein instillation.
Outcome measures	• Fluorescein breakup time (TBUT) • Non-invasive Keratograph® breakup time (NIKBUT) • Area under ROC curve of TBUT, NIKBUT in detecting dry eye. • Youden-optimal diagnostic cut-off sensitivity and specificity of TBUT and NIKBUT in detecting dry eye.
Statistical plan	Non-parametric adjusted power calculations showed that a minimum of 31 participants was required in each of the two participant groups, with and without dry eye. The distributions of NIKBUT and TBUT measurements will undergo D'Agostino-Pearson omnibus normality testing and logarithmic transformation before further parametric analysis if necessary. Intra-group and inter-group comparisons of the medians of TBUT and NIKBUT will be conducted using paired and unpaired t-tests, and the F-test for variances will be performed. Bland-Altman analysis will be conducted between TBUT and NIKBUT time measurements, and Pearson's correlation analysis between TBUT, NIKBUT, and OSDI. ROC curves analysis and Youden-optimal cut-off sensitivity and specificity of TBUT and NIKBUT in detecting dry eye will be reported.

Title: Comparative evaluation of clinical methods of tear film stability assessment: a randomised crossover study

Investigators: Associate Professor Jennifer P. Craig (Principal Investigator)
Michael T. M. Wang (Co-investigator)

1. Introduction and Rationale

Dry eye disease is a common chronic ophthalmic condition affecting more than 20% of the adult population in some parts of the world.[1] It is recognised to have adverse impacts on vision, ocular comfort, and quality of life.[2] Tear film stability is shortened irrespective of dry eye aetiology, and the measurement of tear film breakup time is an integral component of diagnostic testing. [3,4]

In the clinical setting, tear film stability is commonly assessed following aqueous sodium fluorescein instillation, which enhances the visibility of the tear film under blue light, thus simplifying the detection of tear film breakup.[5-7] However, it has been established that the quantity of aqueous fluorescein instilled by conventional clinical methods can destabilise the tear film and reduce breakup time measurements.[8,9]

Recently, automated non-invasive tear film stability measurement techniques have been developed, allowing for the objective assessment of the tear film in its undisturbed state. The Oculus Keratograph® 5M is a commercially available instrument which measures non-invasive tear film breakup time through automated detection of distortion in the contours of reflected placido disc mires, based on real time image analysis. The non-invasive measurement technique may potentially be superior to the conventional fluorescein method through avoiding the destabilising effects of aqueous fluorescein instillation.[8,9] However, the comparative discriminative ability of the non-invasive keratograph and conventional fluorescein tear film breakup time in differentiating between dry eye patients from healthy subjects is not known.

2. Study Objectives

The aim of this randomised crossover study is to compare clinical tear film stability measurements obtained from an automated non-invasive instrument (Oculus Keratograph® 5M, Germany) and the conventional fluorescein method, and to evaluate their discriminative ability in differentiating dry eye patients from normal subjects.

3. Study Design

This study is a prospective, investigator-masked, randomised crossover study evaluating clinical tear film breakup time measurements obtained from the non-invasive Keratograph® 5M and conventional fluorescein methods. Participants will be recruited into two equal-sized age, gender, and ethnicity-matched groups, with and without symptomatic dry eye. The Ocular Surface Disease Index questionnaire will be administered at enrolment, and a cut-off score of ≥13 will be used for the purposes of dry eye classification.

The tear film breakup time of participants will be assessed in triplicate, with and without sodium fluorescein instillation, in a randomised order, by two independent observers to ensure investigator masking. A 30-minute interval between measurements will ensure for subsidence of any reflex tearing.

In the intervention arm, sodium fluorescein will be applied to the bulbar conjunctiva from a fluorescein impregnated strip (Haag-Streit) wetted with 1 drop (10µl) of saline and shaken to

remove excess fluid. Following fluorescein instillation, participants will be instructed to blink naturally over a 1 minute period to facilitate even distribution over the ocular surface. Tear film breakup time will then be assessed in triplicate under blue light with a Wratten yellow filter.

In the control arm, tear film breakup time will be measured using an automated non-invasive measurement technique (Oculus Keratograph® 5M, Germany) without sodium fluorescein instillation.

4. Participant Enrolment

4.1. Power Calculations

Power calculations were conducted using PASS 2002 (NCSS Statistical Software LLC, Utah, USA). The uniform non-parametric adjusted calculations accounted for the randomised crossover study design of the study. It showed that a minimum of 31 participants was required in each of the two participant groups, with and without dry eye, to detect a clinically significant breakup time difference of 5 seconds, with 80% power ($\beta = 0.2$), at a two-sided statistical significance level of 5% ($\alpha = 0.05$). The SD of normal values was estimated to be at 7 s [12].

4.2. Eligibility Criteria

Subjects must meet the following criteria to be eligible for inclusion in the study:

- Age of 18 years or older.
- No history of uncontrolled major systemic disease known to affect the eye (e.g. uncontrolled severe systemic allergy, autoimmune or immunodeficiency disease).
- No history of ocular surgery (such as refractive or cataract surgery) in either eye during the preceding 3 months.
- No use of topical or systemic medications known to cause ocular drying (e.g., cyclosporine, antihistamines, tricyclic antidepressants, anxiolytics, antimuscarinics, diuretics, phenothiazines, steroids, etc.) during the preceding 3 months.
- Able to provide written informed consent.

4.3. Informed Consent

Prior to study enrolment, participants will be provided with a participant information sheet and consent form and be given the chance to review and ask questions before written informed consent is obtained. The participant will sign and date the informed consent form. The original will be retained with the principal investigator, independently of the data, and a copy will be provided to the participant.

5. Study Visit Description

Participants will be required to attend a single 3-hour visit at the Ocular Surface Laboratory. Enrolment eligibility will be assessed after written informed consent has been obtained from the participant. The Ocular Surface Disease Index questionnaire will then be administered. Fluorescein tear film breakup time and non-invasive keratograph breakup time measurements will then be conducted in a randomised order, with a 30-minute interval between measurements to ensure subsidence of reflex tearing.

6. Statistical Analysis Plan

6.1. Normality Assessment

The positively skewed nature of tear film stability measurements has been reported consistently in the literature.[8-10] The distributions of tear film breakup time measurements will therefore be assessed using the D'Agostino-Pearson omnibus normality test, and undergo logarithmic transformation before further parametric analysis if necessary. All statistical tests will be two-tailed and $p<0.05$ considered significant.

6.2 Primary Analysis

Intra-group and inter-group comparisons of the medians of fluorescein and non-invasive tear film break up time will be conducted using paired and unpaired t-tests.

6.3. Secondary Analysis

- The overall distribution of the two tear film stability measurements will be compared using the F-test for variances.
- Pearson's correlation analysis between the two tear film stability measurements and the Ocular Surface Disease Index will be performed.
- Bland-Altman analysis will be conducted between the two tear film breakup time measurements.[11]
- Receiver operative characteristic curves will be constructed to assess the discriminative ability of the two breakup time measurements in differentiating between dry eye patients from normal subjects. The area under the curve (C-statistic) and Youden-optimal diagnostic cut-off sensitivity and specificity will then be calculated.

7. Ethical and Regulatory Compliance

7.1. Good Clinical Practice Statement

It is the responsibility of the study investigators to ensure that the clinical study is conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and are consistent with the International Conference on Harmonisation (ICH) guidelines for Good Clinical Practice (GCP), and all regulatory and institutional requirements, including those for patient privacy, informed consent, human participants ethics committee approval and record retention.

The investigator will also ensure that participants undergo no procedures other than those described in this protocol and approved by University of Auckland Human Participants Ethics Committee (UAHPEC), except those procedures deemed necessary to protect the health and well-being of participants. Any such procedures will be documented in the patient's records and reported to the UAHPEC. If any additional requirements are imposed by UAHPEC, these requirements shall be followed.

7.2. Informed Consent

The principles of informed consent described in the International Conference on Harmonisation (ICH) guidelines for Good Clinical Practice (GCP) will be followed.

Eligible subjects may only be included in the study after providing written (witnessed, where required by law or regulation), IEC-approved informed consent, or, if incapable of doing so, after such consent has been provided by a legally acceptable representative of the subjects. The participant or participant's legally authorized representative will be allowed sufficient time to thoroughly read (or have read and explained to them), the informed consent form. The investigator will answer any questions that the patient/representative might have. Informed consent must be obtained before conducting any study-specific procedures (i.e., all of the procedures described in the protocol). It should be noted in the patient's CRF, with the date, that the informed consent form was obtained prior to any study-related activities being conducted.

The investigator will retain source documents for each subject in the study. The investigator will also retain the original informed consent form signed by subject, and a copy will be given to the participant for their records.

7.3. Subject Confidentiality and Data Protection

The investigator will take all appropriate measures to ensure that the anonymity of each study subject is maintained. The personal data of study participants will be treated in compliance with all applicable legal, regulatory, and institutional requirements.

7.4. Institutional Review Board

A properly constituted Institutional Review Board/Independent Ethics Committee/Research Ethics Board will review and approve:

- The protocol, the proposed informed consent form and participant information sheet, before study commencement.
- Any amendment or modification to the study protocol, informed consent form and participant information sheet before study commencement, unless the change is necessary to eliminate an immediate hazard to study participants, in which the Institutional Review Board/Independent Ethics Committee/Research Ethics Board will be informed as soon as possible.

Prior to study commencement, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the study in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to Institutional Review Board/Independent Ethics Committee/Research Ethics Board and regulatory authorities as required.

8. Protocol Amendments

The investigator will not implement a change in the design or operation of the study protocol, proposed informed consent form and participant information sheet without an Institutional Review Board/Independent Ethics Committee/Research Ethics Board approved amendment.

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by the Institutional Review Board/Independent Ethics Committee/Research

Ethics Board. Only amendments that are required for subject safety may be implemented prior to Institutional Review Board/Independent Ethics Committee/Research Ethics Board approval.

9. Study Documentation

9.1. Retention of records

The investigator is responsible for maintaining adequate records including:

- The Participant Information Sheet, protocol and amendments;
- Signed and dated informed consents per institutional policy;
- Signed, dated, and completed CRFs;
- Notification of SAEs and related reports;
- Dated and documented Ethics Committee approvals and relevant correspondence
- Curriculum vitae and current practising licenses of the principal investigator and clinically qualified co-investigators;

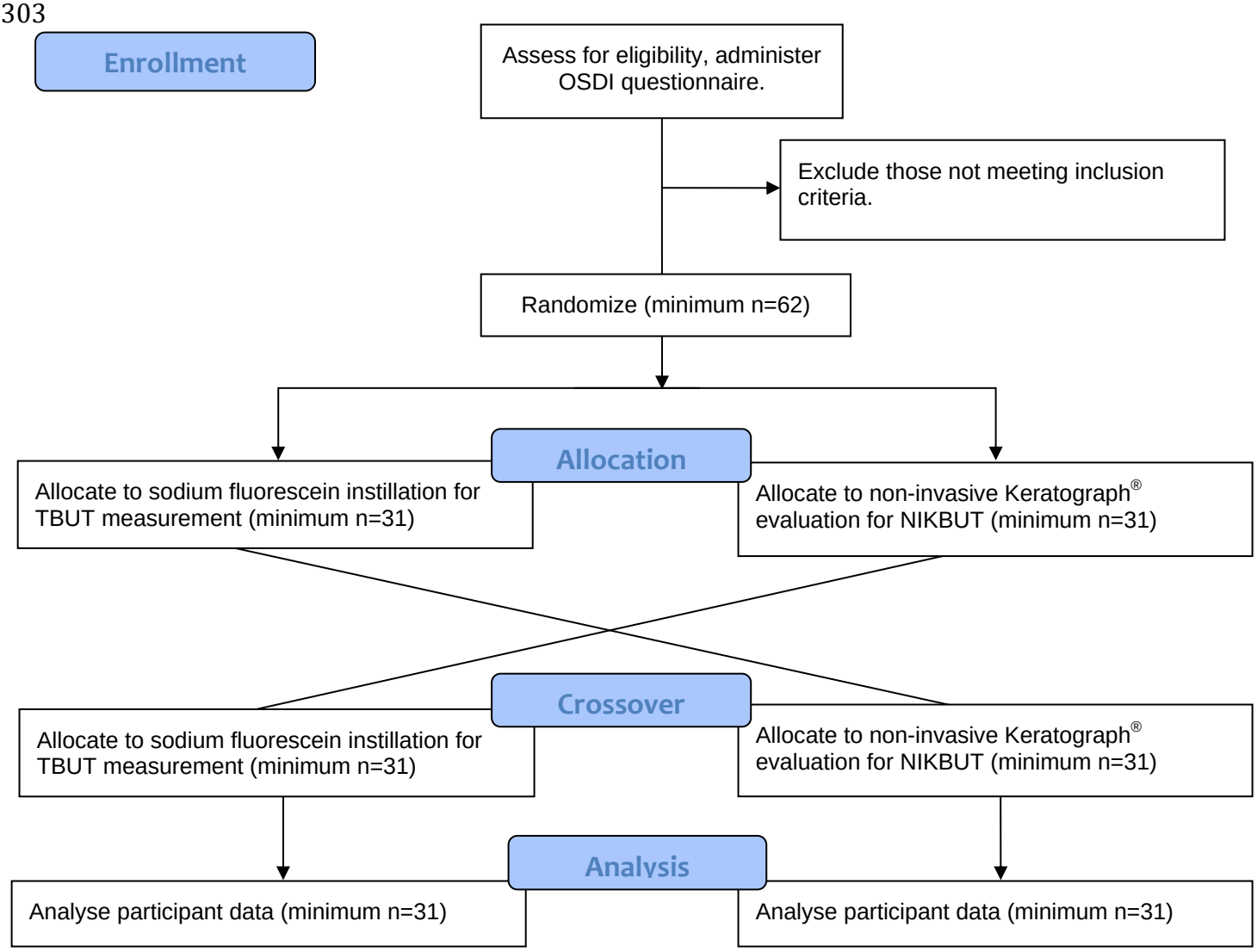
All records will be retained for 6 years after study completion and de-identified electronic data will be retained indefinitely, as per the University of Auckland Human Participants Ethics Committee (UAHPEC) approval. Written records may be retained for a longer period if required by relevant regulatory authorities. Records will be destroyed in a manner that ensures confidentiality.

10. References:

1. The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007). *The ocular surface.* Apr 2007;5(2):93-107.
2. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). *The ocular surface.* Apr 2007;5(2):75-92.
3. Methodologies to diagnose and monitor dry eye disease: report of the Diagnostic Methodology Subcommittee of the International Dry Eye WorkShop (2007). *The ocular surface.* Apr 2007;5(2):108-152.
4. Nichols KK, Mitchell GL, Zadnik K. The repeatability of clinical measurements of dry eye. *Cornea.* Apr 2004;23(3):272-285.
5. Korb DR. Survey of preferred tests for diagnosis of the tear film and dry eye. *Cornea.* Jul 2000;19(4):483-486.
6. Nichols KK, Nichols JJ, Zadnik K. Frequency of dry eye diagnostic test procedures used in various modes of ophthalmic practice. *Cornea.* Jul 2000;19(4):477-482.
7. Smith J, Nichols KK, Baldwin EK. Current patterns in the use of diagnostic tests in dry eye evaluation. *Cornea.* 2008;27(6):656-662.
8. Patel S, Murray D, McKenzie A, Shearer DS, McGrath BD. Effects of fluorescein on tear breakup time and on tear thinning time. *American journal of optometry and physiological optics.* Mar 1985;62(3):188-190.
9. Mengher LS, Bron AJ, Tonge SR, Gilbert DJ. Effect of fluorescein instillation on the pre-corneal tear film stability. *Current eye research.* Jan 1985;4(1):9-12.
10. Craig JP, Tomlinson A. Importance of the lipid layer in human tear film stability and evaporation. *Optometry and vision science : official publication of the American Academy of Optometry.* Jan 1997;74(1):8-13.
11. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet (London, England).* 1986;1(8476):307-310.

Appendix A: Study Design

Adapted from CONSORT flow diagram (http://www.consort-statement.org/consort-statement/flow-diagram).



Appendix B: Clinical Measurements

Ocular Surface Disease Index (OSDI)

Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. *Arch Ophthalmol*. 2000;118:615-621.

OSDI© = (Sum of scores) × 25 ÷ (No. of questions answered)

Have you experienced any of the following during the last week?	All of the time	Most of the time	Half of the time	Some of the time	None of the time
1. Eyes that are sensitive to light?	4	3	2	1	0
2. Eyes that feel gritty?	4	3	2	1	0
3. Painful or sore eyes?	4	3	2	1	0
4. Blurred vision?	4	3	2	1	0
5. Poor vision?	4	3	2	1	0

Have problems with your eyes limited you in performing any of following during the last week?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
6. Reading?	4	3	2	1	0	N/A
7. Driving at night?	4	3	2	1	0	N/A
8. Working with a computer or bank machine (ATM)?	4	3	2	1	0	N/A
9. Watching TV?	4	3	2	1	0	N/A

Have your eyes felt uncomfortable in any of following situations during the last week?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
10. Windy conditions?	4	3	2	1	0	N/A
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	N/A
12. Areas that are air conditioned?	4	3	2	1	0	N/A

Non-Invasive Keratograph® Break Up Time (NIKBUT)

Assessments will be conducted using automated non-invasive Oculus Keratograpj® 5M (K5M) readings:

- The participant's head will be positioned so that their forehead is against the forehead bar and chin is in the chin-rest
- The instrument will be aligned with the central cornea while the participant observes the red dot within the device.
- On aligning and focussing the instrument according to the on-screen instructions, the participant will be requested to blink twice, then to refrain from blinking during measurement.
- The time to first break-up will be recorded by the K5M device
- This procedure will be repeated three time and the three values transferred to the CRF.
- An average value will be calculated for the purpose of analysis.

Fluorescein Break Up Time (TBUT)

Assessment will be conducted using a slit lamp biomicroscope:

- Sodium fluorescein will be applied to the bulbar conjunctiva from a fluorescein impregnated strip (Haag-Streit) wetted with 1 drop (10µl) of saline and shaken to remove excess fluid.
- Following fluorescein instillation, participants will be instructed to blink naturally over a 1 minute period to facilitate even distribution over the ocular surface.
- The participant's head will then be positioned so that their forehead is against the forehead bar and chin is in the chin-rest of the slit lamp biomicroscope.
- The blue light with a Wratten yellow filter setting of the slit lamp biomicroscope will be used.
- On aligning and focussing the instrument, the participant will be requested to blink twice, then to refrain from blinking and to look in the neutral gaze position during measurement.
- The investigator will record the time taken to observe first break-up in the tear film as observed through the slit lamp biomicroscope
- This procedure will be repeated three time and the three values transferred to the CRF.
- An average value will be calculated for the purpose of analysis.