

## **The TearLab Osmolarity System**

### **How does the collection technique affect osmolarity readings?**

Collecting a 50 nanoliter sample of tear fluid, a quantity that is not readily visible to the naked eye, is controlled and monitored by the Test Card lab-on-a-chip technology and the TearLab Pen. Data submitted to the FDA for CLIA Waiver categorization demonstrates that by following the simple user instructions, tear collection will not affect the osmolarity reading.

### **Does it matter where in the tear lake the tear is collected? Will different locations generate different results?**

TearLab recommends that tears be collected from the inferior lateral tear meniscus. Keep in mind that the tear fluid in a dry eye patient has lost homeostatic control and is unstable, with the potential to change from blink to blink. Collecting in the lower lateral cul-de-sac allows the tear fluid to re-mix between blinks and represents the best overall indication of the osmotic state of the tear fluid at the time of collection. In addition, using the higher of either the OD or OS reading, mitigates the unstable effect in a dry eye subject and will provide the correct result with nearly a 90% confidence rate.

### **How much tear fluid does TearLab collect and how is it collected?**

The single-use Test Card contains a microfluidic channel that is gently placed on the tear lake (meniscus) in the corner of the eye on the inner lower lid margin, and via passive capillary action, less than 50-nanoliters of tear sample is instantly and automatically collected when it comes in contact with tear fluid. A sample smaller than the period at the end of this sentence.

### **What is the number on the top of the Test Card used for?**

It is a calibration code that needs to be entered into the Reader after the sample has been collected and the Pen is docked into the Reader. Although every Test Card in a box will have the same calibration code, this number can vary from 1-20 and may vary from box to box. You should take care when opening a new box of Test Cards to ensure that you are entering the correct number when performing a test. Entering the incorrect code will result in an inaccurate test result.

### **Why is the Test Card for single-use only?**

The solutes and biological components from the previous tear fluid sample will remain in the microfluidic channel and will result in an inaccurate measurement if used more than once. Due to the small size of the microfluidic channel, the Test Card cannot be cleaned after use.

The TearLab Test Card is a highly precise component, and even the smallest trace of residual salts will affect subsequent measurements. You should never collect tears with a used Test Card.

### **When I insert a Test Card that has already been used, the light illuminates green indicating it's okay to collect tears.**

The Pen detects whether there is fluid within a Test Card, and if it detects fluid the green light will not illuminate to protect against re-use. If a Test Card is allowed to completely dry out, a used Test Card may illuminate the Pen; however, the Test Card will retain solutes and biological components from the previous tear fluid collection and result in an inaccurate measurement if used more than once. You should never collect tears with a used Test Card. A Test Card that is outside of its foil wrapper that does not have a protective cover on it should be considered used, even if the Pen's green light illuminates. The rule of thumb is to never place a Test Card without a plastic cover on a Pen. A Test Card without a plastic cover should not be used to collect tears. Test Cards should be discarded immediately following use to avoid their accidental reuse.

### **What does CV mean?**

CV (Coefficient of Variation) is an industry standard measurement of precision equal to the standard deviation divided by the mean of a set of measurements. The smaller the CV the more precise and accurate the test. The TearLab has a CV of less than 2%, which is considered excellent and comparable to the most sophisticated laboratory technologies. In addition, such a "tight" CV indicates that the TearLab instrument is capable of measuring osmolarity with little or no variability between consecutive measurements of a single fluid sample, and any variability seen in patient measurements is related to the instability of the tear film due to loss of homeostatic control in the dry eye disease state. Thus, the variability between measurements in a normal, healthy lacrimal system will not exceed the TearLab CV of 2%

in patient measurements. The TearLab instrument has thus allowed us to identify and quantify instability as a hallmark of the disease, and this variability in homeostatic control can now be used as a confirming clinical indication of disease and if correctly understood, should not confound the clinician who encounters variability of measurements. Any difference between OD and OS measurements greater than 8 mOsm/L is a classic indication of loss of homeostatic control and is a confirming indicator of dry eye disease.

### What is the difference between sensitivity and specificity and positive predictive value (PPV) and negative predictive value (NPV)?

Sensitivity is the ability to diagnose persons who actually have the disease.

Specificity is the ability to diagnose persons who do not have the disease.

Positive Predictive Value is the percent of people with a positive test who have the disease.

Negative Predictive Value is the percent of people with a negative test who do not have the disease.

In laboratory medicine the reliability of a binary or dichotomic (yes/no; healthy/sick) test is generally evaluated in terms of sensitivity and specificity, while in non-binary tests (where there is a severity scale - such as in dry eye, ie., normal, mild, moderate, severe), these terms are less relevant. The most important measure in a diagnostic method is the Positive Predictive Value, as it reflects the probability that a positive test reflects the underlying condition being tested. In clinical studies, tear osmolarity consistently demonstrates a high PPV (98.4% by Versura et al; Curr Eye Res. 2010 Jul;35(7):553-64).

### How does TearLab perform relative to the historical data of osmometers?

The TearLab Osmolarity System has demonstrated performance equivalent to existing laboratory osmometers. The historical performance of osmolarity in published literature is compared to published data on TearLab performance. What is significant is that TearLab, for the first time, provides a clinical tool that meets this performance criteria outside of the laboratory environment, at the point-of-care in the hands of the doctor office staff.

(ref: BD Sullivan et al. "Diagnostic performance of osmolarity combined with subset markers of dry eye disease in an unstratified patient population," ARVO 2010.).

	Historical Performance of Osmolarity Laboratory Staff	TearLab Osmolarity System Doctor Office Staff
<b>Sensitivity</b>	69%	72%
<b>Specificity</b>	92%	92%

### **Osmolarity and Dry Eye Disease (DED)**

#### What data supports the use of tear film osmolarity for the diagnosis of DED?

The place of tear osmolarity measurement in the diagnosis of dry eye disease is "well-established" in guidelines and physician-authored, peer-reviewed literature published over many years: (Methodologies to diagnose and monitor dry eye disease: Report of the Diagnostic Methodology Subcommittee of the International Dry Eye WorkShop. *The Ocular Surface* 2007;5:108-123.)

- *AAO Cornea/External Disease Panel (2011)*: The American Academy of Ophthalmology (AAO) Preferred Practice Pattern for Dry Eye Syndrome specifically recommends tear osmolarity testing for the diagnosis and management of dry eye syndrome. Note that the TearLab tear osmolarity test is identified as "a more sensitive method of diagnosing and grading the severity of dry eye compared to corneal and conjunctival staining, tear

break-up time, Schirmer test and meibomian gland grading". Please note that this was a change in the 2011 Preferred Practice Pattern compared with the 2010 Preferred Practice Pattern and reflects the Academy's determination that the TearLab test is well-established and not investigational. (American Academy of Ophthalmology. Preferred practice pattern: Dry eye syndrome. 2011)

- *AOA Optometric Clinical Practice Guideline* (2010): The American Optometric Association (AOA) clinical practice guideline for "Care of the Patient with Ocular Surface Disorders" identifies the "tear osmolarity test" as appropriate for use in evaluating the dry eye state including lacrimal gland function and the quality of the precocular tear film. (American Optometric Association. Clinical practice guideline: Care of the patient with ocular surface disorders. 2010.)
- *The Dry Eye WorkShop* (2007): The Dry Eye WorkShop (DEWS) Consensus Standard for Diagnosis and Classification of Dry Eye Disease identifies "increased osmolarity" as a component in the definition of dry eye disease. (The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop. *The Ocular Surface* 2007;5:75-92.)
- *Lemp et al.* (2011): In this prospective, observational case series intended to assess the clinical usefulness of tear osmolarity and other common tests to diagnose dry eye disease, the authors concluded that tear osmolarity "is the best single metric both to diagnose and classify dry eye disease". (Lemp MA, Bron AJ, Baudouin C, Benitez del Castillo JM, et al. Tear osmolarity in the diagnosis and management of dry eye disease. *Am J Ophthalmol* 2011;111:792-798.)
- *Jacobi et al.* (2011): In this paper the authors describe a study of 133 patients with moderate to severe dry eye disease. They report a mean tear osmolarity of 320 mOsmol/L in the disease group as opposed to 301 mOsmol/L in the healthy control group. The sensitivity was 87% and the specificity was 81%. They conclude, "testing tear film osmolarity can be a very effective objective diagnostic tool in the diagnosis of dry eye disease." (Jacobi C, Jacobi A, Kruse FE, Cursiefen C. Tear film osmolarity measurements in dry eye disease using electrical impedance technology. *Cornea*. 2011; 30:1289-92.)
- *Sullivan et al.* (2010): In this prospective, multisite clinical study intended to evaluate the clinical utility of tests for assessing dry eye disease severity, the authors concluded that tear film osmolarity "was found to be the single best marker of disease severity across the normal, mild/moderate, and severe categories". (Sullivan BD, Whitmer D, Nichols KK, Tomlinson A, et al. An objective approach to dry eye disease severity. *IOVS* 2010;51:6125-6130.)
- *Versura et al.* (2010): In this study intended to assess the diagnostic performance of tear osmolarity compared to other clinical and laboratory tests for dry eye disease, the authors concluded that tear osmolarity "showed a good performance in dry eye diagnosis, higher than the other tests considered, mainly in severe dry eye". (Versura P, Profazio V, Campos EC. Performance of tear osmolarity compared to previous diagnostic tests for dry eye diseases. *Curr Eye Res* 2010;35:553-564.)
- *Khanal et al.* (2008): In this study intended to determine the most effective objective test(s) for the diagnosis of dry eye disease, the authors concluded that "tear osmolarity is the best single test for the diagnosis of dry eye, whereas a battery of tests employing a weighted comparison of TTR [(tear turnover rate)], evaporation, and osmolarity measurements derived from discriminant function analysis is the most effective." (Khanal S, Tomlinson A, McFadyen A, Diaper C, et al. Dry eye diagnosis. *IOVS* 2008;49:1407-1414.)
- *Tomlinson et al.* (2006): In this meta-analysis performed on published data for tear osmolarity in samples of normal eyes and various subtypes of dry eye, the authors concluded that "measurement of tear film osmolarity arguably offers the best means of capturing, in a single parameter, the balance of input and output of the lacrimal system." (Tomlinson A, Khanal S, Ramaesh K, Diaper C, et al. Tear film osmolarity: determination of a referent for dry eye diagnosis. *IOVS* 2006;47:4309-4315.)

### How does osmolarity correlate to Dry Eye Disease?

Normal tear film is in homeostasis with blood osmolarity at a reference value of 290 mOsm/L, with a normal "homeostatic range" for tear osmolarity defined as 275-307 mOsm/L. It is important to note that both clinical and sub-clinical hyperosmolar stress to the ocular surface, above the normal homeostatic range, has been related to:

- Cell death (apoptosis)
- Inflammation
- Loss of lubrication
- Fluctuation in vision

These factors contribute to a progressive pathogenesis commonly referred to as Dry Eye Disease (DED).

Normal osmolarities are typically found at a mean value of 302 mOsm/L, mild/moderate osmolarity (Dry Eye) at a mean of 315 mOsm/L and severe osmolarity (Dry Eye) at a mean of 336 mOsm/L. Osmolarity values in the low end of the abnormal range should be considered together with symptoms for a definitive dry eye diagnosis. Values in the upper range of normal (for the general population) in the presence of symptoms should suggest early/mild DED.

### Does osmolarity correlate to symptoms of Dry Eye Disease?

In DED, it is known that symptoms correlate poorly with objective signs. It is important to note that in the absence of symptoms the presence of hyperosmolarity above the normal homeostatic range (275 - 307 mOsm/L) is still evidence of DED. Hyperosmolarity in the absence of symptoms should be considered an abnormality in the body's homeostatic osmoregulation and a sign of DED.

### Does osmolarity increase over disease progression?

DED is a chronic and progressive disease. The TearLab Osmolarity System can aid in the diagnosis of early and late stage disease. Abnormal (or Dry Eye) values can increase to the mild/moderate range (with a mean value of 315 mOsm/L) to the severe range (with a mean value of 336 mOsm/L). Studies are currently ongoing to establish the relationship between disease progression and increasing osmolarity values.

### Is there a relationship between osmolarity and the known classifications of DED? E.g. will a Meibomian Gland Dysfunction patient have a higher osmolarity than a patient with Lacrimal Gland Dysfunction?

The osmolarity value will be a composite of the combined effects of either dysfunction (aqueous deficiency or evaporative) and should be used in a global assessment of dry eye disease. The ultimate endpoint of disease progression is a hyperosmolar state.

### Why is osmolarity considered to have a causative effect in addition to being a physical sign of DED?

Hyperosmolarity plays a role in the physical destabilization of the tear film, damage to the ocular surface and contributes to the progression of the disease. It is recognized as both a sign of the disease and the central causative mechanism in the disease pathogenesis.

### What is the relationship between systemic hyperosmolarity and ocular hyperosmolarity?

There is no known relationship between the osmolarity of other body fluids and tear fluid. However, studies are currently ongoing to better define this relationship.

## TearLab in Practice

### How should the TearLab Osmolarity System be used in clinical practice?

The TearLab Osmolarity System is intended to measure the osmolarity of human tears to aid in the diagnosis of dry eye disease in patients suspected of having dry eye disease, in conjunction with other methods of clinical evaluation.

### Is it necessary to test both eyes?

Osmolarity may differ from left and right eye (and this is particularly characteristic in patients with DED). Each eye should be tested and the higher osmolarity should be considered the relevant value; look for other signs of DED with

other methods of clinical evaluation. (Ref: B.D. Sullivan et al., "Diagnostic performance of osmolarity combined with subset markers of dry eye disease in an unstratified patient population", ARVO 2010).

Test Result	Action
> 308 mOsms/L in either eye	Consider treating for dry eye disease
< 308 mOsms/L in both eyes	Consider other pathologies or other methods of clinical evaluation

### What do variations in readings between eyes in the same patient mean?

The ability to mitigate environmental stress via compensatory mechanisms will vary from eye to eye within the same patient, thus resulting in variations in the osmolarity readings. As dry eye disease progresses, osmolarity readings may vary from eye to eye, with at least one eye in the abnormal range.

Patients with dry eye disease (hyperosmolarity) have a dysfunctional tear film, combined with poor homeostatic osmoregulatory capability. As such, stress on the tear film causes instability that is not seen in normal tear film. The TearLab instrument allows us to quantify eye-to-eye variability and can be used as a confirming clinical indication of disease and, if correctly understood, should not confound the clinician who encounters variability in measurements. Any difference between OD and OS measurements greater than 8 mOsm/L is a classic indication of loss of homeostatic control and is a confirming indicator of dry eye disease.

This is demonstrated in the table below, in which four consecutive TearLab osmolarity measurements are made one-minute apart over three days in both a dry eye and normal patient.

\* As seen below, the dry eye patient has wide fluctuations in tear osmolarity, eye-to-eye, test-to-test and day-to-day, attributed to the pathological instability of the tear film, while the normal patient shows no variability beyond the expected analytical variability of the instrument itself.

	Mild/Moderate Dry Eye OSDI = 22.92		Normal Patient OSDI = 4.17	
	Right Eye	Left Eye	Right Eye	Left Eye
<b>Day 1</b>				
1 min	311	326	286	288
2 min	304	324	285	289
3 min	308	308	281	281
4 min	337	334	287	286
<b>Day 2</b>				
1 min	315	321	296	284
2 min	305	313	296	291
3 min	315	323	285	291
4 min	297	343	291	287
<b>Day 3</b>				
1 min	308	307	290	292
2 min	320	312	287	291
3 min	307	309	286	286
4 min	333	332	292	295
<b>Mean</b>	<b>313</b>	<b>321</b>	<b>289</b>	<b>288</b>
<b>Stdev</b>	<b>11.8</b>	<b>11.5</b>	<b>4.6</b>	<b>3.9</b>

### Can I expect a patient to have consistent readings from one visit to the next?

No. Just as other physiological indicators, such as intra-ocular pressure or blood pressure, vary day-to-day and measurement-to-measurement, tear film osmolarity will also vary. In the demonstration chart above, diagnosed dry eye patients were tracked over a three-day period and tested. As indicated previously, the dry eye patient has wide fluctuations in osmolarity while the normal subject had basically no biological variability.

### Are there diurnal variations in osmolarity readings?

Currently there is no data to support diurnal variations. Studies are ongoing to address this question.

### Are there factors that affect how I should evaluate osmolarity within different demographic groups?

TearLab is not currently aware of any factors that affect how osmolarity should be evaluated within different demographic groups.

### In DED patients, should I perform several readings and derive a mean as a baseline before treatment?

Hyperosmolarity (> 308 mOsm/L) defines dry eye disease in conjunction with clinical evaluation and a single measurement can result in a treatment decision. In which case, a mean is not necessary to establish diagnosis. Test both eyes and take the higher of the two readings, regardless of which eye, for the clinical assessment of that patient at that time point.

### My patient has all the signs of DED, but I keep getting low osmolarity results.

Osmolarity should be considered in conjunction with other methods of clinical evaluation.

In the presence of other signs and symptoms of DED such as pronounced corneal staining, or short breakup times, consider that the patient may be exhibiting an osmolarity at the low end of the abnormal range. Also ensure that the patient is not using eye drops within one hour of testing, and also consider that if the patient is being treated, that the treatment may be reducing the tear osmolarity.

In the absence of other signs, if a patient exhibits consistently low osmolarity over time, consider pathologies other than dry eye disease.

### Should patients with high osmolarity be excluded as candidates for LASIK surgery?

TearLab osmolarity is intended to diagnose DED in patients suspected of having Dry Eye Disease in conjunction with other methods of clinical evaluation. Literature suggests that pre-treating patients diagnosed with DED prior to LASIK surgery results in better refractive outcomes following LASIK surgery.

### How do systemic medications affect osmolarity?

Numerous systemic medications may result in side effects. Some systemic medications may change the make up of the tear film, which may increase ocular dryness, possibly resulting in a higher osmolarity. TearLab currently makes no claims for the effect of medication on osmolarity.