Reprinted from CORNEA
Vol. 33 No. 8 August 2014

Prospective, Multicenter, Clinical Evaluation of Point-of-Care Matrix Metalloproteinase-9 Test for Confirming Dry Eye Disease

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Purpose: The aim of this study was to determine the negative and positive agreement of a point-of-care matrix metalloproteinase-9 test in confirming the diagnosis of dry eye and to evaluate the ease of use by untrained ophthalmic technicians.

Methods: The study was a prospective, sequential, masked, clinical trial with 4 clinical trial sites. The InflammaDry test was compared with the clinical assessment of tear break-up time, Schirmer tear testing, and corneal staining for the confirmation of dry eye, both with and without the inclusion of the Ocular Surface Disease Index (OSDI), as a confirmatory test.

Results: The study enrolled 237 patients. If the OSDI is included in the definition for mild dry eye, the InflammaDry test was shown to have a total positive agreement of 81% (127/157) and a negative agreement of 98% (78/80). The removal of the OSDI shifted the categorization of 11 patients previously considered positive for dry eye to become categorized as negative for dry eye. If the OSDI is excluded from the definition of dry eye, the InflammaDry test demonstrates a positive agreement of 86% (126/146) and a negative agreement of 97% (88/91) against the clinical assessment.

Conclusions: The InflammaDry test demonstrates a high positive and negative agreement for confirming suspected dry eye disease. In addition, the test was safely and effectively performed by untrained operators. These findings support the intended use of the InflammaDry test as an aid in the diagnosis of dry eye.

Received for publication January 8, 2014; revision received April 12, 2014; accepted May 3, 2014. Published online ahead of print June 25, 2014.
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The design of the study and collection of data were sponsored by Rapid Pathogen Screening Inc (RPS). No sponsorship was received for the analysis and interpretation of study data. R. Sambursky has an affiliation with RPS but was not an investigator. The remaining authors have no funding or conflicts of interest to disclose.
Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01692964.
Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.corneajrnl.com).
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Key Words: dry eye, matrix metalloproteinase, MMP-9, inflammation, ocular surface, clinical study

(*Cornea* 2014;33:812–818)

According to the Dry Eye Workshop (DEWS) report, dry eye is a multifactorial disease of tears and the ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface.[1] It is also accompanied by increased osmolarity of the tear film and inflammation of the ocular surface.[1] Dry eye is affected by the relationship between the amount of tears produced, rate of tear evaporation, and the presence or absence of inflammation.

Symptoms alone are inadequate for the diagnosis of dry eye, because the same symptoms can be experienced with a range of ocular surface conditions and tear film disorders. Additionally, both symptoms and signs can vary greatly depending on the environmental conditions to which patients are exposed in their daily lives. Only 57% of symptomatic patients have been shown to have objective signs of dry eye.[2–6] This finding has been attributed to the symptoms preceding the signs, or the differing etiology and pathophysiology of dry eye.[7]

The most common objective diagnostic test for dry eye, the Schirmer tear test, has been in use for >100 years.[8] The Schirmer tear test lacks standardization[9] and is inaccurate and unrepeatable because of the reflex secretion produced by its irritating nature.[10] This test is limited to the measurement of tear production,[11] while overlooking the evaporative aspects of dry eye.[9] The discomfort, time inefficiency, and lack of sensitivity associated with Schirmer tear testing further limit its use. However, the low cost of strips and ease of application has led the Schirmer tear test to become the most common clinical test for lacrimal secretary function in dry eye.

Tear break-up time (TBUT) measurement with fluorescein is another widely used technique for the clinical diagnosis of dry eye. TBUT is considered to be more reliable than the Schirmer test, because it is repeatable and minimally invasive; however, the instillation of a topical anesthetic can destabilize the tear film and lead to an artificially accelerated TBUT.[12–14] Further, all forms of tear break-up measurement fail to give direct information on tear production.[9] Because support staff assess most patients before the clinician's evaluation, the ability to accurately perform TBUT or corneal staining before the application of a topical anesthetic is limited by patient flow.

Ocular surface staining with vital dyes such as Rose Bengal, lissamine green, and fluorescein has also been used to diagnose dry eye disease.[15–17] The disadvantage of staining is that dry eye cannot be clinically differentiated from other conditions that lead to ocular surface staining such as medication toxicity (including topical anesthetic), poor lid apposition, underlying infection, or trauma.[18] Additionally, these staining techniques are not likely to be used in early dry eye or mild dry eye.[18]

Outside the research setting, the majority of dry eye–affected patients encounter an ophthalmic technician as part of the initial patient work-up. Typically, patients report on the presence of symptoms. The Ocular Surface Disease Index (OSDI) is a questionnaire that was developed to identify and quantify the common symptoms associated with dry eye as a means to measure the therapeutic effect of a dry eye medication.[19] The OSDI consists of 12 questions, each scored by the patient. This assessment has been found to be subjective, to lack specificity, and to be prone to operator-dependent analytical errors, preventing it from routine clinical use.[1,20–22]

Increased osmolarity has been described in patients having dry eye,[23] because reduced tear secretion and/or increased evaporation results in the loss of fluid that isotonic tears cannot overcome.[24] Elevated tear osmolarity is considered an important indicator of dry eye. Specifically, normal tear osmolarity is understood to be reflected by tears in the range of 275 to 307 mOsm/L, whereas hyperosmolarity is indicated by a tear fluid osmolarity $\geq$308 mOsm/L in 1 or both eyes, or a $>$8 mOsm/L difference in tear osmolarity between the eyes.[25]

Versura et al[26] measured tear osmolarity in 25 normal subjects and in 105 dry eye–affected patients and found that the normal values were 296.5 $\pm$ 9.8 mOsm/L. Values were shown to increase as dry eye severity increases (mild to moderate to severe dry eye, respectively: 298.1 $\pm$ 10.6 vs. 306.7 $\pm$ 9.5 vs. 314.4 $\pm$ 10.1, $P < 0.05$).[26] In a study performed by Lemp et al,[25] osmolarity was determined to be more sensitive than other clinical signs. In this study, the most sensitive threshold between normal and mild or moderate subjects was found to be 308 mOsms/L, whereas the most specific threshold was found to be at 315 mOsms/L.[25] At a cutoff of 312 mOsms/L, tear hyperosmolarity exhibited 73% sensitivity and 92% specificity.[25] In another study performed by Tomlinson et al[27], a cutoff value of $>$316 mOsm/L, derived from the distribution of osmolarity values, was used to diagnose dry eye disease with an effectiveness of 73% sensitivity, 90% specificity, and 85% positive predictive value. Osmolarity measurements have been shown to vary between sample measurements, and reflect the inherent tear film instability of dry eye disease.[28,29]

Matrix metalloproteinases are proteolytic enzymes that are produced by stressed epithelial cells on the ocular surface. Specifically, matrix metalloproteinase-9 (MMP-9) is an inflammatory marker that has consistently been shown to be elevated in the tears of patients with dry eyes. Elevated MMP-9 levels in patients with moderate to severe dry eye disease correlate with clinical examination findings.[30] Altered corneal epithelial barrier function is the cause for ocular irritation and visual morbidity in dry eye disease. MMP-9 seems to play a physiological role in corneal epithelial desquamation. Although MMP-9 does not provide information about tear production, increased MMP-9 activity in dry eye may contribute to deranged corneal epithelial barrier function, increased corneal epithelial desquamation, and corneal surface irregularity.[30]

MMP-9 activity is also elevated in ocular surface conditions such as blepharitis and Sjögren syndrome; however, these underlying conditions lead to the development of inflammatory dry eye disease that would be accurately detected using the InflammaDry test.[31] Other conditions such as infection,[32] allergy,[33] pterygium,[34] and conjunctivalchalasis,[33] have been associated with an elevated level of MMP-9, but are readily clinically differentiated from dry eye disease. According to an analysis performed by Sambursky and O'Brien,[35] normal levels of MMP-9 (nanograms per milliliter) in human tears range from 3 to 40 ng/mL.

The aforementioned characteristics and limitations of dry eye diagnostic tools suggest that diagnosis of this multifactorial disease may be improved upon with a protocol inclusive of multiple diagnostic tools (Fig. 1). A new single use, noninvasive, inexpensive, disposable test that can accurately aid in the confirmation of the diagnosis of dry eye, such as InflammaDry, provides valuable information without imposing infrastructure challenges. InflammaDry will cost less than the test's anticipated reimbursement. Using direct sampling microfiltration technology, the InflammaDry immunoassay detects elevated levels of MMP-9 ($\geq$40 ng/mL) in tears to confirm the diagnosis of dry eye disease.

The InflammaDry test was evaluated in a prospective, multicenter, masked clinical trial to determine the negative and positive agreement of the test in confirming the diagnosis of dry eye disease. The term positive agreement is used in lieu of sensitivity when reporting the performance data of a diagnostic test against which there in no single diagnostic gold standard to compare. Similarly, the term negative agreement is used in lieu of specificity when reporting the performance data of a diagnostic test against which there in no single diagnostic gold standard to compare. The clinical trial took place over a 7-month period and used untrained ophthalmic technicians (operators) at 4 clinical sites representing a combination of academic and private practices.

MATERIALS AND METHODS

Study Design

The study design was a prospective, sequential, masked, clinical trial. Those patients who were clinically determined by an ophthalmic clinician to meet enrollment criteria were included in the study (see Table, Supplemental Digital Content 1, http://links.lww.com/ICO/A230).

Institutional review board approval was first obtained. A subject's participation was limited to a 1-time event that occurred at the time of specimen collection. There were no follow-up visits necessary for this study. The subjects did not incur any costs associated with the study procedures. Before starting the clinical trial, each site conducted positive and negative external controls on the InflammaDry test to confirm the functionality of the test reagents.

DRY EYE DIAGNOSTIC PROTOCOL

Initial Visit

NOTE: The InflammaDry test may be performed independent of other testing. If TearLab osmolarity testing is performed, it must be performed before InflammaDry or any other testing. TearLab test results may be negatively impacted by reflex tearing. Reflex tearing does not affect InflammaDry test results. However, InflammaDry must be performed before the installation of any drops.

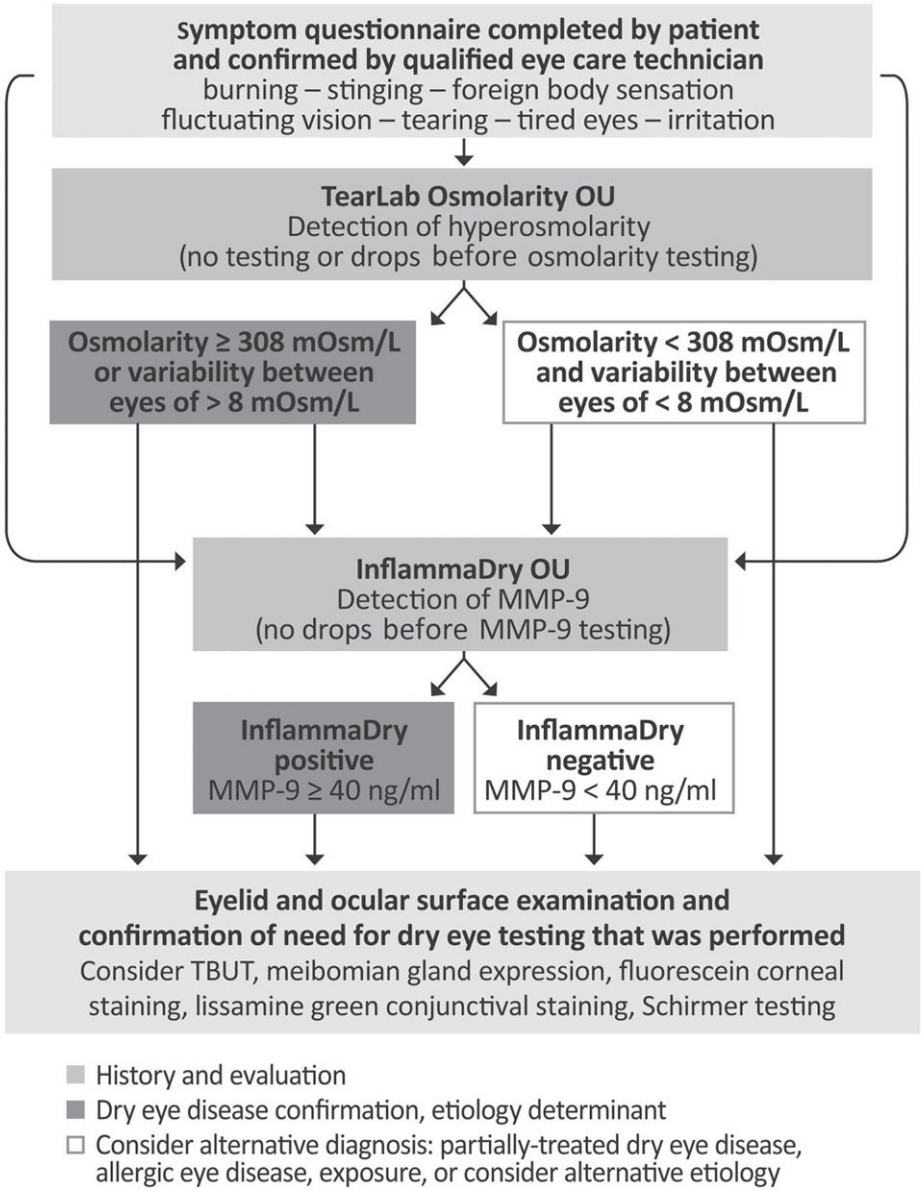

FIGURE 1. Dry eye diagnostic protocol, initial visit.

At the office visit, before any study-related procedures, the subjects were screened through a standard of care history and a slit-lamp examination. After determining that the patient qualified for enrollment into the study, an investigator or delegated study personnel obtained an informed consent. Upon obtaining the subject's consent, study personnel interviewed the subject and documented data, including the subject's age, gender, race, and patient history, on a sponsor-provided Case Report Form.

Study Visit Testing

Study testing was done on the subject's more symptomatic eye. If no difference existed, the right eye was tested.

InflammaDry

The ophthalmic technician (operator) performing the InflammaDry test was limited to the manufacturer's instructions for use as their only resource. In addition, the untrained operator was unaware of the patient's clinical history and did not perform or learn of any subsequent test results including the OSDI survey, TBUT, corneal fluorescein staining, or the Schirmer tear test.

First, to perform the InflammaDry test, the untrained operator collected a tear sample from the patient's palpebral conjunctiva. The operator then gently dabbed the provided sample collector in multiple locations along the palpebral conjunctiva; they released the lid after every 2 to 3 dabs to allow the patient to blink. This was repeated 6 to 8 times, and then the sampling fleece was allowed to rest against the conjunctiva for at least 5 seconds or until the sampling fleece was saturated with tears (5–10 μL). Adequate saturation of the sampling fleece was indicated by a pink color or glistening appearance. Next, the test was assembled by snapping the sample collector onto the provided test cassette. The assembled test was then dipped into the provided test buffer solution for 20 seconds for activation. Last, after 10 minutes had elapsed, the test values were read. The presence of 1 blue line and 1 red line in the test result window indicate a positive test result (MMP-9 $\geq$ 40 ng/mL). The intensity of the red line is directly related to the amount of MMP-9 present; thus, mild dry eye is associated with fainter lines than more severe dry eye is. The presence of a red line of any intensity confirms the presence of elevated MMP-9. One blue line indicates a negative test result (MMP-9 $<$ 40 ng/mL). All InflammaDry tests were analyzed within 24 hours of activation.

Per the trial protocol, invalid test results were to be documented and reported to show usability, but these results were not to be included in any positive or negative agreement calculations. There were no invalid test results in this study. The InflammaDry test has built-in procedural controls, including a blue control line. In the unlikely event that the test is not run properly or the reagents do not work, the blue control line will not appear, indicating an invalid test result.

Ocular Surface Disease Index

To evaluate patient-reported symptoms associated with dry eyes, the OSDI survey was completed. OSDI scores range from 0 to 100, where 0 indicates no disability and 100 indicates complete disability.

Fluorescein Tear Break-up Time

The TBUT was evaluated 2 minutes after the inferotemporal bulbar conjunctiva was touched with a 1-mg sodium fluorescein strip (wet with preservative-free saline). Subjects were instructed to blink, and the precorneal tear film was examined under blue-light illumination with a biomicroscope and 10× objective. The interval between the blink and the appearance of the first dark spot or discontinuity in the precorneal fluorescein-stained tear layer was then recorded. Three separate readings were taken for each eye, and the results were averaged.

Corneal Fluorescein Staining

The ocular surface was also examined 2 minutes after fluorescein instillation into the tear film as described above. The Oxford grading scheme was used to grade the intensity of corneal fluorescein staining in 5 different zones of the conjunctiva and cornea (central, superior, temporal, inferior, and nasal). The result was based on the number of dots on a 5-point scale: no dot = 0; 1 to 5 dots = 1; 6 to 15 dots = 2; 16 to 30 dots = 3; and $>$30 dots = 4. Additionally, if there was 1 area of confluence, 1 point was added. Two points were added if there were $\geq$2 areas of confluence or if filamentary keratitis was present.

Schirmer Tear Test

Topical anesthetic was then introduced into the inferior fornix. The Schirmer tear test was performed by placing Schirmer test strips (Tear Flo, Alta Loma, CA) over the lower lid margin, at the junction of the lateral and middle thirds, for 5 minutes. The strip wetting was measured and recorded in millimeters. If complete wetting of the strips occurred before 5 minutes, and if the person administering the Schirmer tear test felt that an initial response occurred because of reflex tearing, then it was documented and the test was repeated after measures had been taken to prevent reflex tearing (ie, reanesthetizing the eye, removing any potential irritants, and waiting a few minutes). If, after every effort to prevent reflex tearing, a similar complete wetting of the strips occurred before 5 minutes, then the result was documented and accepted.

Clinical Assessment and Dry Eye Disease Severity

The InflammaDry test was compared with the clinical assessment as specified in Table 1. Derived from the DEWS criteria, the clinical assessment was developed to represent a combination of symptoms and signs. The clinical trial used the same metrics for TBUT, Schirmer tear testing, and corneal staining as described in the DEWS criteria. However, conjunctival injection, conjunctival staining, and the presence of meibomian disease were not tested or used to characterize the severity of dry eye disease. In general, the worst severity for

TABLE 1. Dry Eye Disease Severity Grading

Clinical Testing	Negative Control	Mild Grade 1	Moderate Grade 2	Moderately Severe Grade 3	Severe Grade 4
OSDI score*	<13	≥13	≥13	≥13	≥13
TBUT, s	>10	≤10	≤10	≤5	0 (Immediate)
Staining (0–5)	0 (None)	0 (None)	1–2	3	≥4
Schirmer, mm/5 min	>10	≤10	≤10	≤5	≤2

*Study data were analyzed with and without the inclusion of the OSDI as a confirmatory test for dry eye.

any sign tested determined the overall severity. Patients were categorized to the highest severity level at which all required criteria were satisfied. Patients who did not meet all the required clinical criteria for a given severity grade were considered to be at the next lower grade.

Sample Size Justification and Statistical Significance

The study concluded with a total of 237 patients, 146 in the dry eye group and 91 in the control group. Using a binomial 1-sided test against 75% that was significant at the 0.05 alpha level, the sample size of 146 patients provided >90% power to test against a null hypothesis of 75% positive agreement. In the control group, the sample size of 91 patients provides 83% power to detect a negative agreement of at least 75%. The InflammaDry test demonstrated a positive agreement of 86% (126/146) with a P value of <0.0001 and 95% confidence interval of 0.80 to 0.91 and a negative agreement of 97% (88/91) with a P value of <0.0001 and 95% confidence intervals of 0.91 to 0.99.

RESULTS

The study enrolled 237 patients consisting of 164 women and 73 men between the ages of 18 and 94 years with a mean age of 53 years. The categorization of dry eye severity was analyzed with and without the inclusion of the OSDI as a confirmatory criterion for the presence of dry eye disease. Eleven patients were found to have an elevated OSDI without any objective confirmatory testing as shown in Table 2.

Table 3 demonstrates the categorization of dry eye–affected patients enrolled based on the signs they had presented, with and without the inclusion of the OSDI as a confirmatory criterion for the presence of dry eye. The removal of the OSDI shifted the categorization of 11 patients previously considered positive for dry eye to become categorized as negative for dry eye.

Table 4 demonstrates the performance of the InflammaDry test against the clinical assessment that both includes and excludes the OSDI as a confirmatory test for the presence of dry eye. If the OSDI is included in the definition for mild dry eye, the InflammaDry test was shown to have a total positive agreement of 81% (127/157) and a negative agreement of 98% (78/80). If the OSDI is excluded from the definition of dry eye, the InflammaDry test demonstrates an 86% (126/146) positive agreement and a 97% (88/91) negative agreement against clinical assessment as an objective confirmatory criterion for the presence of dry eye.

TABLE 2. OSDI Discordance from Dry Eye Confirmatory Testing

No. Subjects	OSDI	TBUT	Schirmer Tear Test	Staining
11	≥13	>10	>10	0

DISCUSSION

According to the American Academy of Ophthalmology's Preferred Practice Pattern for dry eye disease, many ocular surface diseases produce symptoms that are similar to those associated with dry eye. Although it is useful to identify characteristics of the symptom causative factors, such as adverse environments, prolonged visual efforts, or ameliorating circumstances, tests are required to confirm the diagnosis of dry eye disease. The 2 major factors that contribute to dry eye independently, deficient aqueous tear production and increased evaporative loss, may also be present together.[1]

The preferred practice pattern states that no single test is adequate for establishing the diagnosis of dry eye and recommends a combination of TBUT, Schirmer tear testing, and staining as the current gold standard. For mild disease, a rapid TBUT may indicate an unstable tear film, whereas aqueous tear deficiency may be diagnosed with the Schirmer tear test. Minimal or no dye staining of the ocular surface may exist with mild dry eye disease.

The InflammaDry test was compared with clinical assessment, defined as the presence of subjective symptoms of suspected dry eye accompanied by at least one of the following objective confirmatory clinical signs: reduced Schirmer tear test, reduced TBUT, or the presence of corneal staining. Each of these objective measurements provides distinct information about the condition of the ocular surface. The trial was performed at 4 clinical sites. Similar to the data by Chotikavanich et al,[30] MMP-9 was found to be elevated over the entire range of dry eye severity (Table 3). Moreover, MMP-9 was consistently elevated in patients with mild dry eye despite the lack of corneal staining in study participants with mild dry eye (Table 1).

Three out of the 4 clinical sites had 4 different untrained ophthalmic technicians perform the testing, whereas 1 site used only 1 untrained ophthalmic technician to enroll all their patients. Two clinical sites, using a total of 8 different untrained technicians, enrolled >74% of the patients. These sites demonstrated the best performance despite having the

TABLE 3. Patient Dry Eye Severity Grading With and Without OSDI

Confirmatory Testing With and Without OSDI Inclusion*	No Dry Eye Grade 0	Mild Grade 1	Moderate Grade 2	Moderately Severe Grade 3	Severe Grade 4
Without OSDI	91	49	76	20	1
InflammaDry positive	3	47	60	18	1
With OSDI	80	60	76	20	1
InflammaDry positive	2	48	60	18	1

*Study data were analyzed with and without the inclusion of the OSDI as a confirmatory test for dry eye.

greatest number of untrained technicians participating. Of the 2 remaining sites, only 1 site enrolled a significant number of patients (21%), but this clinical site used only a single untrained technician to perform all the testing, and this site accounted for the majority of the reported false negatives observed in the study. The remaining clinical site enrolled <5% (12 patients). Taken together, the analysis showed that nearly 90% of the untrained technicians were effective at running and interpreting the test, whereas 1 operator accounted for most of the false negative results.

There is a general trend for patients to report more severe dry eye symptoms relative to the clinical signs observed by their clinician.[36] Symptoms have been shown to be insufficient for the diagnosis and management of dry eye; thus, a consensus of clinical signs is recommended for the diagnosis of dry eye.[2] A study conducted by Amparo et al[37] showed no correlation between changes in patient-reported symptoms using OSDI and changes in tear osmolarity or corneal fluorescein staining. A comparable study conducted by Caffery et al[38] showed no significant correlation between tear osmolarity and the self-assessment of dry eye in a nonclinical population of 249 convention attendees.

Similarly, if the OSDI is included as a confirmatory test, the positive and negative percent agreements of the InflammaDry test changes. In this trial, inclusion of the OSDI results lead to 11 patients being potentially falsely characterized as having dry eye disease despite the lack of any demonstrable objective signs. These results suggest that the OSDI may be a good screening tool for identifying patients with symptoms consistent with dry eye but is not suitable as a confirmatory diagnostic test.

The InflammaDry clinical trial was designed as an all comers trial where all patients enrolled reported at least 1 symptom consistent with dry eye over the preceding month. Only approximately two-thirds of patients with symptoms consistent with dry eye disease tested positive with any objective confirmatory test, including TBUT, Schirmer tear testing, or corneal staining. Of those who tested positive with any objective confirmatory test, approximately 85% tested positive with InflammaDry. These results suggest that 50% of all symptomatic patients and nearly all of those confirmed as dry eye have significant ongoing inflammation. Similarly, a study reported by McDonald shows that less than half of all symptomatic subjects (42.8%), symptomatic cataract-affected patients (48.9%), and symptomatic laser-assisted in situ keratomileusis–operated subjects (42.7%) had actual dry eye disease as tested by tear osmolarity (unpublished data submitted for presentation at the American Society of Cataract and Refractive Surgeons 2014 Symposium).

Dry eye is a multifactorial, chronic disease and inflammation occurs in most, but not in all, patients with dry eye. Another possible explanation for the discordance between dry eye symptoms and both hyperosmolarity and elevated MMP-9 may be the intermittent nature of mild dry eye disease, which leads to symptoms only at the time of an environmental stress. These patients would most likely demonstrate a higher rate of signs if tested at that time. Thus, mild dry eye could be thought of as intermittent moderate disease, differentiated primarily by the temporal frequency of symptoms.

Because inflammation is found throughout the lacrimal unit, MMP-9 levels are unlikely to be affected by reflex tearing.[31] A study on relative humidity by Tesón et al[39] demonstrated that MMP-9 levels increase in the presence of low relative humidity. However, additional studies are needed to assess the variability of MMP-9 levels in dry eye–affected patients.

The reported clinical study supports the use of MMP-9 as a marker for dry eye and the InflammaDry test as a clinical aid in the diagnosis of dry eye disease. Additionally, the

TABLE 4. Performance Results of MMP-9 Test Compared with Confirmatory Testing

N = 237		Clinical Assessment + OSDI + TBUT + Schirmer + Staining		Positive % Agreement 95% CI	Negative % Agreement 95% CI
InflammaDry	Positive	127	2	81% (127/157) (74%, 87%)	98% (78/80) (91%, 100%)
	Negative	30	78		
		Clinical Assessment + TBUT + Schirmer + Staining			
InflammaDry	Positive	126	3	86% (126/146) (80%, 91%)	97% (88/91) (91%, 99%)
	Negative	20	88		

clinical performance demonstrated by untrained ophthalmic technicians in this trial correlates with that reported in a previous prospective clinical trial by Sambursky et al[40] in which investigators at 7 clinical sites determined an InflammaDry test sensitivity of 85% (121/143) and specificity of 94% (59/63).

Because InflammaDry is a qualitative test, it is not designed or intended to monitor the disease state after the initiation of treatment. However, several investigators have suggested that a combination of clinical variables, including the measurement of surface epitheliopathy/staining, along with various biomarkers such as MMP-9, may be the most reliable prognosticator for response to therapy.[37] Therefore, identifying symptomatic dry eye–affected patients with underlying inflammation may guide patient management and therapeutic recommendations, including artificial tear replacement, punctal occlusion, or antiinflammatory therapeutics such as a short course of corticosteroids, oral doxycycline, or long-term maintenance treatment with cyclosporine.

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