VERSÉA™ OPHTHALMICS T-POC TOTAL IgE IMMUNOASSAY KIT

INTENDED USE

The VERSÉA™ OPHTHALMICS T-POC TOTAL IgE IMMUNOASSAY KIT is an in vitro diagnostic device that is used for the quantitative determination of Immunoglobulin E (IgE) concentration in human tears. It is also used as an aid in the diagnosis of ocular allergies including Type 1 allergic conjunctivitis or the allergic component of ocular inflammatory responses.

SUMMARY AND EXPLANATION OF THE TEST

Approximately 1/3 of the global population is affected by ocular surface disease (OSD) which most commonly results from either, or a combination of, ocular allergies and dry eye disease. Ocular allergies (OA) symptoms occur in 40% to 80% of affected individuals.[1] Dry eye disease (DED) and OA often mimic each other and frequently coexist.[2] Despite the fact that DED and OA are different ocular conditions, there is significant clinical overlap between the two diseases. OA and DED can be considered as predisposing or, at least, facilitating conditions to each other, or co-existing conditions.[2] Moreover, severe presentations of OA are challenging to differentiate from infectious conjunctivitis. Patients with atopic allergic diseases such as atopic asthma, atopic dermatitis, allergic rhinitis, allergic conjunctivitis and hay fever have been shown to exhibit increased total Immunoglobulin E (IgE) levels in blood.[3,4,5] In general, elevated levels of IgE indicate an increased probability of an IgE-mediated hypersensitivity, responsible for Type 1 (anaphylactic) allergic reactions.

Certain groups of white blood cells, including basophils and tissue mast cells, have membrane receptors for the IgE molecule. Type I reactions are triggered when an allergen attaches to two adjacent IgE molecules bound to the surface of the mast cell or circulating basophil. Target cells, through a series of complex reactions, form a combination of a specific allergen with antibody-sensitized basophils or mast cells, and initiate the release of certain vasoactive agents, such as histamine.[6,7,8] Locally (as in ocular allergy), IgE is produced at the conjunctival site by the local plasma cells that line the substantia propria.[9] It has been documented that IgE positive cells are present in the subconjunctival layer in all cases of allergic conjunctivitis during the exacerbation phase of the disease.[10] In these cases, the capacity of IgE to bind to receptors on mast cells and basophils, and the subsequent binding of antigen to the bound IgE molecules on these cells, also leads to the release of histamine and other soluble mediators, which produces the symptoms referred to as "allergies."[11] It has been shown that IgE synthesis can be induced by the interaction of B cells with mast cells and basophils in the presence of interleukin-4.[12] The conclusion is that inflammatory cells have a very important role in regulating IgE production at local levels in reactions such as allergic conjunctivitis. As a result of these biochemical mediators, there is a constriction of smooth muscles, dilation of small blood vessels, activation of blood platelets, and irritation of nerve endings characteristic of allergic reactions. Typical clinical symptoms of immediate hypersensitivity are inflammation and itching in a skin reaction, or congestion in a bronchial reaction or, locally, ocular hyperemia and itching.

In general, the concentrations of IgE in a particular patient appear to be related to both the extent of the allergic reaction that occurs, and cumulative number of different allergens to which the individual responds. Studies have reported that locally produced IgE is the source of the allergic condition in allergic conjunctivitis.[13,14] For allergic conjunctivitis, while local concentrations of IgE may be high, systemic concentrations may be low except in vernal allergic conjunctivitis (indicating both a systemic and a local response). Type 1 (anaphylactic) IgE mediated allergic responses include: GAC (General Allergic Conjunctivitis) which includes SAC, PAC and AKC:

SAC (Seasonal Allergic Conjunctivitis) -- This includes hypersensitivity to dust, pollens, microbes and drugs. It is sometimes referred to as hay fever conjunctivitis, and can be associated with allergic rhinitis.

PAC (Perennial Allergic Conjunctivitis) -- This includes hypersensitivity to dust and animal dander of pets (which may be relatively constant factors in the home environment).

AKC (Atopic Keratoconjunctivitis) -- This includes chronic bilateral keratoconjunctivitis associated with atopic dermatitis.

Other ocular IgE mediated allergic responses include:

GPC (Giant Papillary Conjunctivitis) -- This includes hypersensitivity reaction to antigens trapped on the surface of a contact lens, exposed suture or prosthesis.

VKC (Vernal Keratoconjunctivitis) -- This is a bilateral hypersensitivity associated with systemic allergic responses. Frequently, this condition has a high level of serum IgE as well as high levels of tear IgE.[15]

The most common ocular atopy is allergic conjunctivitis, the ocular counterpart of allergic rhinitis. Exposure to environmental allergens such as pollens, animal dander and dust cause the symptoms and signs of ocular hay fever in sensitized individuals. Allergic conjunctivitis is a Type 1 allergic reaction caused by immunoglobulin E (IgE).[16,17] The diagnosis of allergic conjunctivitis is based mainly on clinical features, particularly itching, and hyperemia or the presence of papillary formation on the conjunctiva. However, there is no one diagnostic technique that can be used reliably to confirm the presence of OA.[18] Eosinophils in conjunctival scrapings are helpful in confirming the diagnosis,[19-25] but positive detection rates are not very high.[26,27] Abelson, et al. found eosinophils in only 45% of their patients.[25]

Hoffmann, et al.[28] note that OA is a common symptom among Type I (IgE mediated) allergic diseases. They found that the correlation between symptoms of OA and the presence of allergen-specific IgE in tears was highly significant (p>0.0001). In contrast, they found only a poor correlation (p = 0.73) between specific and/or total IgE antibodies in sera and the manifestation of OA. They conclude that allergen-specific IgE antibodies in tears seem to be produced locally rather than exudated from serum and that IgE in tears seems to be responsible for allergic conjunctivitis. They believe that this conclusion emphasizes the diagnostic value of identifying tear IgE values when diagnosing allergic conjunctivitis.

IgE is considered to play an important role in allergic reactions in the eye.[29] Diagnosis of OA has been attempted by measurement of serum IgE concentrations.[30] However, measurement of serum IgE concentrations does not differentiate between local and systemic levels.[30] It is important to be able to detect local allergic reactions so that they can be used to distinguish locally produced allergic conjunctivitis.[31-33] Locally produced IgE has been shown to be the largest contributor to the severity of the disease.[20,21] Thus, tear IgE measurements could provide a much better way to diagnose allergic conjunctivitis.

Donshik and Ballow[34] showed that the tear IgE levels are significantly increased in giant papillary conjunctivitis (GPC). GPC is a well-recognized clinicopathologic entity associated with the wearing of contact lenses. GPC is characterized by papillary changes of upper tarsal conjunctiva, erythema, increased mucous production and decreased contact lens tolerance. Ballow and Donshik[35] similarly showed dramatic increases in the amounts of IgE found in tears from patients with vernal conjunctivitis (VKC). Moreover, tear IgE levels have been found to be higher in the more symptomatic eye of an individual with GPC.[36] This is consistent with the fact that allergic insults are considered cumulative.

Insler, et al.[37] measured the concentration of IgE in the tears of allergic patients. In comparing the mean tear IgE levels between groups of patients, a statistically significant difference was found between the allergic symptomatic patients versus nonallergic asymptomatic patients. This study also found statistically higher levels of IgE for the allergic asymptomatic group versus the nonallergic asymptomatic group. These patients may have had a sub-acute level of allergic response.

Mathers, et al.[38] looked at a model for ocular tear film function. To assess the relationships between tear osmolarity, tear flow, evaporation, and lipid function, they studied a series of normal patients and those with blepharitis, dry eye, and blepharitis, DED, and OA and measured a set of variables associated with the production and maintenance of the tear film. The authors construct a model which shows that knowledge of the tear flow and lipid parameters leads to a better understanding of DED. This model (based on their experimental results) suggests that lipid disruption due to ocular allergy probably affects evaporation and may alter tear flow. This means that an allergic eye could disguise itself as a presenting DED.

OA has been shown to be associated with changes in tear film composition that leads to tear film instability, which facilitates the ocular allergic process to last longer and recur.[31] Eosinophilic activation and concomitant release of inflammatory mediators may damage the conjunctival epithelial and goblet cells. As a result, the alteration and deficiency of the mucin layer cause instability of the tear film. Changes in the tear film lipid layer that are typical of DED and a significantly thicker tear film lipid layer have been found in SAC patients without corneal fluorescein staining suggesting that the instability of the tear film was caused by the alterations of the lipid layer[39] (Li 2010).

Tear IgE varies from 159 IU/ml to less than 1 IU/ml compared with controls of 8 IU/ml to less than 1 IU/ml.[29] Nomura et al[40] sampled tears from patients with allergic conjunctivitis, bacterial conjunctivitis (BC), epidemic keratoconjunctivitis (EKC) and normal controls and determined that their tear IgE concentrations showed significant increases in allergic conjunctivitis when compared with controls and no significant difference was found between EKC and BC and controls.

Several authors[41-44] confirmed the extensive overlap in clinical signs and symptoms associated with various ocular disease states such as OA, DED, and infectious conjunctivitis and confirm that redness and/or itching are not exclusively associated with OA.

Yang et al.[43] studied allergic conjunctivitis as a risk factor for regression and haze after photorefractive keratectomy (PRK). The groups of patients were (a) normals, (b) patients treated for allergic conjunctivitis and (c) no-treatment allergic conjunctivitis group. The mean corneal haze score for the no-treatment allergic group was significantly higher than the treatment or normal groups, which were not different from each other. A refractive outcome of + 1 diopter was obtained for 100% of normals, 93.8% for the treatment group and 36.4% for the no-treatment group. The authors suggest that untreated OA is a significant risk factor for haze and myopic regression after photorefractive keratectomy. Monitoring tear IgE levels could aid in pre-surgical screening of PRK candidates.

Following treatment of OA, symptoms improved as the concentration of total IgE in tears decreased.[44] The difference between the total IgE concentrations in tears measured at the first visit and the first follow-up visit positively correlated with the difference between the total clinical scores recorded at subsequent two visits. Similarly, there was a significantly positive correlation between the change in the tear total IgE concentration and the difference in clinical score at every 2-week follow-up. Total IgE in the tears of patients who improved within treatment for 7 days was significantly lower than that in the tears of the patients who improved after treatment for 7 days and total IgE concentrations in tears at the initial visit were significantly higher in patients who relapsed after drug discontinuation than in patients who had recovered. Patients at risk for recurrent disease show total IgE concentration in tears of patients with recurrent illness is significantly higher than that in patients without recurrent disease. Higher tear total IgE concentration at the initial visit was also associated with a longer delay in improvement following treatment and may predict the course of treatment by monitoring changes in the tear total IgE concentration.[44]

PRINCIPLE OF THE TEST

The T-POC TOTAL IgE IMMUNOASSAY KIT is a lateral flow immunoassay device using mouse monoclonal antibody to human IgE coupled to gold particles as the conjugate, with the membrane coated with mouse anti-human IgE monoclonal antibody as the solid phase. With the addition of a tear sample, immune complexes are formed by the interaction of IgE in the sample with the conjugated gold nanoparticles. These antibody/IgE complexes flow past the solid phase antibody where they are bound and immobilized. The accretion of gold particles produces a color formation on the solid phase membrane, which is measured by the Reader. If no IgE is present in the tear sample, the conjugated antibody cannot be bound specifically, so little or no color develops. The intensity of color development is directly proportional to the concentration of IgE in the tear sample.

REAGENTS

The VERSÉA™ OPHTHALMICS T-POC TOTAL IgE IMMUNOASSAY KIT contains the following


50 – foil-pouched Test Cassettes
100 – 1.0 µL microcapillary tubes (for sample collection)
1 bulb for connecting to the microcapillary tubes
1 – Dropper bottle filled with 7mL of assay running buffer.
Lateral Flow Reader (not included).

STORAGE INSTRUCTIONS

1. T-POC TOTAL IgE IMMUNOASSAY KIT should be stored at room temperature.
2. The expiration dating indicates the limits of stability. The expiration dating is as indicated on the Kit packaging

ADDITIONAL MATERIALS REQUIRED (available from VERSÉA™ OPHTHALMICS)

The VERSÉA™ OPHTHALMICS LATERAL FLOW READER (LFR)
T-POC TOTAL IgE Validation Kit
T-POC TOTAL IgE External Controls
T-POC TOTAL IgE Proficiency Kit

REFERENCE STANDARDIZATION

The T-POC TOTAL IgE IMMUNOASSAY KIT is standardized using Scripps purified Human IgE which is standardized against WHO 2nd IRP 75/502.

REAGENT PREPARATION

None.

WARNING AND PRECAUTIONS

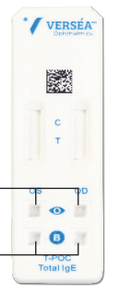
1. The test cassette should remain in the sealed pouch until ready for use.
2. Do not smoke, eat or drink in the areas where specimens or kit components are handled.
3. All specimens, reagents, and controls should be potentially hazardous and handled in the same manner as infectious agents.
4. Wear disposable gloves while handling samples and wash hands after the assay is complete.
5. The test cassette and all materials should be discarded in a proper biohazard container after testing.
6. For *in vitro* diagnostic use only.
7. Materials should not be used after the expiration date shown on the package label.

HEALTH AND SAFETY INFORMATION

WARNING: In accordance with the principles of good laboratory practice (GLP) it is recommended that all samples be treated as if potentially infectious.

TEST CASSETTE

The TEST CASSETTE has two ports for the eye samples to be delivered next to the symbol of an eye (left eye sample on the left (OS) and right eye sample on the right (OD)) and two ports for the activating buffer delivery on either side of the symbol "B".



SPECIMEN COLLECTION

Step 1: Remove TEST CASSETTE from its foil pouch and place it onto a flat horizontal surface.

Step 2: Assemble the tear sampler by inserting supplied glass microcapillary into the associated rubber bulb assembly with about a third of the microcapillary tube visible in the bulb assembly. Stabilize the bulb with glass microcapillary between the thumb and the middle finger, making sure the hole on top of the bulb is not closed with your index finger.

Step 3: Tear sample is obtained from the interior marginal tear strip (near the exterior canthus) with the glass microcapillary tube. The utilization of a slit-lamp or a handheld light may be useful to visualize sample. Pull down the lower eyelid to expose the inferior cul-de-sac. Place the microcapillary tube into the tear lake at the inferior medial region. Tears are drawn into the microcapillary tube by microcapillary action by establishing contact with the tear film. Ensure the microcapillary is completely filled.

Note: The microcapillary tube container has a protective plastic covering under the cap which must be removed before the glass microcapillary tubes may be acquired from their container. Once the plastic is peeled off, replace the cap, and obtain individual microcapillary tubes by gently shaking container and allowing the tubes to emerge through the hole in the cap.

Note: The bulb is reuseable. Use a new microcapillary tube with each tear sampling (one for each eye) and dispose of the microcapillary tube after the tears are transfered to the cassette.

Step 4: After each collection the sample is transferred directly to the sample port of the CASSETTE labeled with a "OS" or "OD", as appropriate. Touch the micropipette to the sample area and dispense the sample by covering the opening in the bulb. Gently squeeze the bulb until the entire sample has been delivered.

TEST PROCEDURE

Step 5. Access the [SCAN] screen on the Reader. The menu will guide you through the correct steps.

Step 6. Step 1-4 Above: As per the recommended "SPECIMEN COLLECTION" procedure, collect a sample from both the left and right eye. After each collection, transfer tear sample directly to the corresponding sample port of the CASSETTE, preferentially to the top half of the port.

Step 7: Add two (2) drops of running buffer to each "B" port, alternating between the two ports.

Step 8: Place the TEST CASSETTE into the READER

Step 9: Slide the tray with the TEST CASSETTE into the READER until it clicks into place and press [TIMED SCAN] button on the touch screen.

Step 10: After 8 min countdown, the Reader displays the result for the left eye under [OS Strip] tab and the result for the right eye under [OD Strip] tab.

- If the control values are valid, the patient value are also acceptable. If the control values are invalid the run is unacceptable. Retest the patient.

Note: Quality Control: U.S. CLIA Regulation requires that 2 controls be run on each day of patient sample testing and when there is a change of lot number. Refer to the corresponding package insert for controls.

External controls are available but not provided with the testing kit. Perform quality control testing per your laboratory protocol. The lab may implement an IQCP protocol. Refer to the laboratory IQCP plan for QC testing frequency.

ASSESSMENT OF RESULTS

To evaluate the patient IgE level, assess the following:

1. The Reader display for the Control must be valid.
2. Quality Control: If the Reader displays a valid Control value, the patient value is also acceptable. If the Control value is not valid, the run is unacceptable; rerun the test samples. Also, Low or High Standards should be successfully assayed prior to each batch of sample testing.
3. If the patient has "Redness and Itching" and the IgE value is < 80 ng/mL, there is a 95.7%

probability that the patient does not have an ocular allergy or an allergic component of the inflammatory response.

4. If the patient has "Redness and Itching" and the IgE value is ≥ 80 ng/mL, there is a 92.9% probability that this elevated IgE is indicative of an ocular allergy or an allergic component of the inflammatory response.
5. Very high IgE levels (>300 ng/mL) may be associated with vernal conjunctivitis and should alert the practitioner to check for systemic etiologies.
6. The IgE results should be used as an aid with other clinically available tools in the diagnosis of ocular allergies or the allergic component of the inflammatory response and is not intended to be the sole diagnostic tool.

PROCEDURAL NOTES

1. Although the T-POC TOTAL IgE IMMUNOASSAY KIT is easy to perform, reliable results will only be obtained when the instructions are followed precisely.
2. TEST CASSETTES may be used only once.
3. A new microcapillary tube should be used for each sample or standard. The microcapillary tube must be full; there should be no bubbles or empty space in the tube.
4. Perform the analysis steps immediately upon adding the buffer solution.

LIMITATIONS OF THE PROCEDURE

1. The T-POC TOTAL IgE test procedure must be closely followed.
2. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice (GLP).
3. The practitioner should consider the results obtained with this KIT as additional information to that obtained from patient history, clinical presentation, other test results, etc. Patients may have elevated IgE levels who are not presenting allergic symptoms, and patients may have normal IgE levels who are demonstrating allergic symptoms. IgE mediated allergic reaction cannot be implicated as the single etiologic agent when IgE levels appear to be elevated, nor should it be ruled out when levels appear to be normal.

HEALTH AND SAFETY INFORMATION

Warning: The "IgE Standards" contain highly purified IgE from human source material found to be negative for HbsAg and HIV. Because no known test method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV), or other infectious agents are absent, all human blood products, including human source material, should be handled in accordance with good laboratory practices using appropriate precautions (e.g. Centers for Disease Control/ National Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", 1998).

The T-POC TOTAL IgE IMMUNOASSAY KIT was used by personnel who had a variety of previous training including the investigators themselves and the office technician or assistant. The technician usually ran the test. Therefore, it can be used in a standard clinical setting under the CLIA guidelines.

PERFORMANCE CHARACTERISTICS

Sensitivity (the ability to correctly identify the allergic state) --- If a patient presents with "Redness and Itching" and the T-POC TOTAL IgE IMMUNOASSAY KIT provides a tear IgE reading ≥80 ng/mL, there is a 92.85% probability that the patient has an allergic conjunctivitis or an allergic component of ocular inflammatory response.

Specificity (the ability to correctly identify the non-allergic state) --- If a patient presents with "Redness and Itching" and the T-POC TOTAL IgE IMMUNOASSAY KIT provides a tear IgE reading <80 ng/mL, there is a 95.7% probability that the patient does not have an allergic conjunctivitis or allergic component of ocular inflammatory response.

Accuracy (Sensitivity + Specificity)/2 --- Accuracy of the T-POC TOTAL IgE IMMUNOASSAY KIT is 94.3%.

Precision --- Using a standard solution of human IgE at 450 ng/mL, the day-to-day precision of the T-POC TOTAL IgE IMMUNOASSAY KIT ranged from 89.4% to 95.7% based on the coefficient of variation for 8 sites. Using the Control solution of human IgE at 200 ng/mL, the day-to-day precision of the T-POC TOTAL IgE IMMUNOASSAY KIT ranged from 95.5% to 98.8% based on the coefficient of variation for 8 sites.

Dynamic Range --- The T-POC TOTAL IgE IMMUNOASSAY KIT can accurately measure human IgE concentrations between 20 and 2000 ng/mL.

Interactions --- Non-specific interactions of tear proteins in the T-POC TOTAL IgE IMMUNOASSAY KIT were determined by spiking samples with the following: IgA, IgG, lactoferrin, albumin, and lysozyme. The data below represents the range of these substances tested, all of which had no observable cross-reaction or interference on the assay's ability to measure IgE.

	Minimum Tested	Maximum Tested	Normal Tear
IgA	0	2 mg/mL	1.6 mg/mL
IgG	0	2 mg/mL	0.02 mg/mL
Lactoferrin	0	2 mg/mL	1.5 mg/mL
Albumin	0	5 mg/mL	3.9 mg/mL
Lysozyme	0	2 mg/mL	1.2 mg/mL

CLINICAL DATA

DUKE UNIVERSITY: Using essentially the same protocol as used for the clinical investigation, Foulks, Baratz, and Ferrone of the Duke University Department of Ophthalmology Cornea Service, did a study involving 111 patients. They quantified levels of IgE in normal patients and patients with external ocular signs and symptoms due to OA and infectious disease using the T-POC TOTAL IgE IMMUNOASSAY KIT. Patients selected were categorized as having seasonal allergic conjunctivitis, prosthetic or contact lens associated giant papillary conjunctivitis and vernal keratoconjunctivitis. Normal patients and patients with viral and bacterial conjunctivitis were also tested. The study of 111 patients included 41 normals, 44 allergic (19 SAC, 15 GPC, 10 VKC), and 26 patients with non-allergic diagnoses.

They concluded that there was a statistically significant result differentiating between normals and patients with GPC, seasonal allergic conjunctivitis and/or vernal keratoconjunctivitis. There was no statistical significance demonstrated between normals and viral, bacterial, or non-specific and diabetic patients.

The final data show that, if 80 ng/mL is used as the threshold, the IgE measurement was statistically tied to the diagnosis of the allergic or conversely the non-allergic state. The data also showed that the IgE measurement for the individual and combined categories of GAC, GPC and VKC vs. Viral and Other was statistically different as was the correlation without the impact of VKC measurement. The statistics also showed that the Duke data was not different from the data collected during the Touch Scientific, Inc. study of clinical utility and is treated as one site for the multi-site analysis. See "IgE Measurement - TSI plus Duke".

TOKYO WOMEN'S COLLEGE: An independent clinical evaluation was performed at the Tokyo Women's College and reported below.[40]

The medical research team used the T-POC TOTAL IgE IMMUNOASSAY KIT to do this study. This study included 113 allergic conjunctivitis patients (70 seasonal allergic patients - SAC, 21 perennial allergic patients - PAC, and 22 vernal keratoconjunctivitis patients - VKC). Also included were 14 bacterial conjunctivitis patients - BC, 13 epidemic keratoconjunctivitis (viral) patients - EKC and 18 normal controls.

The results showed significant increases in tear IgE for VKC, SAC and PAC when compared with controls. No significant difference was found between EKC and BC groups and controls. They further concluded that this method can be used to "distinguish allergic conjunctivitis from other forms of conjunctivitis." Differences were assumed to be significant at p< 0.05.

Type of conjunctivitis	IgE Values (ng/mL)	Type of conjunctivitis	IgE Values (ng/mL)
SAC	195 ± 22	EKC	97 ± 12
PAC	135 ± 23	BC	93 ± 14
VKC	322 ± 46	Normals	52 ± 10

FIELD STUDY OF CLINICAL UTILITY:

The primary assessment was to determine if IgE levels, as measured by the Versea T-POC Total IgE test system, could aid in the differentiation between ocular diagnoses with similar initial clinical presentations of the inflammatory response such as red eye and "grittiness" (i.e. allergic conjunctivitis from conjunctivitis due to bacterial, viral and mechanical irritation)." All IgE values were blinded to the investigators until the end of the study.

Of particular importance to the analysis were those patients for whom "red and itch" both existed. This is because if there is no itch, it has been historically presumed that there is no allergic conjunctivitis. The data was also tested to see if signs and symptoms actually contribute to a differential diagnosis between allergic and non-allergic findings. Using previous studies, the combined data and comparison statistics and ROC plots, it was established that a threshold IgE reading of 80 ng/mL and above would determine that the IgE response represented allergic conjunctivitis.

IgE CONCENTRATIONS

	w/red & itch		w/no red & itch		DUKE Study		Total
	Mean	*n*	Mean	*n*	Mean	*n*	*n*
GAC	180	11			214	19	**30**
GPC					264	15	**15**
Vernal	360	2			778	10	**12**
Viral	25	7	19	1	29	10	**18**
BC	46	9	38	6			**15**
Other	31	7	248	3*	29	16	**26**
TOTALS		**36**		**10**		**70**	**116**
Normals					26	41	**41**
Allergic		13		0		44	**57**
Non-allergic		23		10		26	**59**

*The "other" category is skewed by one reading of 705 ng/mL (toxic). It was not known whether there was an allergic component to the toxic finding. The other two readings were both <20 ng/mL

PATIENTS DEMONSTRATING SIGNS/SYMPTOMS OF RED & ITCH:

Diagnosis without the use of the IgE value: This group of data relates to the clinical diagnosis of all cases of conjunctivitis with the doctor using all the standard signs, symptoms and available clinical tools, except IgE values. Because vernal is easier to diagnose as compared to the ability to differentiate between general allergic and bacterial or viral red eye, the data were analyzed with and without the impact of vernal conjunctivitis (VKC). All IgE values were blinded to the investigators until the end of the study. The study showed that if the category of vernal conjunctivitis (VKC) is excluded, it is difficult to differentiate between allergic conjunctivitis and non-allergic conjunctivitis using conventional methods of clinical diagnosis. In fact, using standard clinical tools, the differential diagnosis is not statistically significant. Differences were assumed to be significant at p<0.05. This means that, for the phase of this study where diagnosis is made without the benefit of the IgE value, the investigators were statistically likely to diagnose a "red and itchy" eye as either allergic or non-allergic with no relation to the actual final true diagnosis.

For the "Red and Itch" patients, both allergic and non-allergic eyes present with redness and itching. The study showed that "Signs and Symptoms" is a statistically unreliable marker for clinical diagnosis of the allergic eye. Statistical significance was assumed at p<0.05.

Diagnosis with the use of IgE value: For General Allergic Conjunctivitis, the differentiation of diagnosis between allergic and non-allergic conjunctivitis using the IgE result is statistically significant and repeatable (p = 0.000).

Results for IgE Clinical Study (Vernal omitted)
IgE information provided to doctors for diagnoses

		IgE >79 Allergic	IgE ≤80 Non-Allergic	Total	
Doctors' Diagnoses	Allergic	11	0	11	Fisher Exact p-value = 0.0000
		100.0%	0.0%		
	Non-Allergic	1	22	23	
		4.4%	95.7%		
	Total	12	22	34	

Primary Effectiveness Analysis (including Vernal)
Final Diagnosis: Allergic n = 13 (92.9%)
Total Findings >79 ng/mL = 14

The data supports the conclusion that IgE values equal to or greater than 80 ng/mL represent the allergic categories of diagnosis. The study statistics further confirm that all categories that are not allergic (bacterial, viral and other) are not different from one another in terms of the measured range of IgE. These data support the conclusion that IgE values below 80 ng/mL represent the non-allergic categories of diagnosis. The data supports the conclusion that IgE values equal to or greater than 80 ng/mL represent the allergic categories of diagnosis. The study statistics further confirm that all categories that are not allergic (bacterial, viral and other) are not different from one another in terms of the measured range of IgE. These data support the conclusion that IgE values below 80 ng/mL represent the non-allergic categories of diagnosis.

CLINICAL CONCLUSIONS

OA is accompanied by elevated tear IgE, with 80 ng/mL as a reliable threshold for using as an aid in the diagnosis of Type I allergic conjunctivitis. Both allergic and non-allergic eyes present with redness and itching. All studies show "Signs and Symptoms" as statistically unreliable for clinical diagnoses of allergic eyes.

Without knowledge of the tear IgE values, the diagnosis for all "Red and Itch" patients showed no statistical difference when comparing GAC vs bacterial, viral or other conjunctivitis (each is a non-allergic category).

With knowledge of the tear IgE values, the final diagnosis for all "Red and Itch" patients was highly statistically different when comparing General Allergic Conjunctivitis vs bacterial, viral or other conjunctivitis (each is a non-allergic category) and using a threshold value for Allergic Conjunctivitis of 80 ng/mL.

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