Review Article

INTERPRETATION OF IgE-MEDIATED ALLERGY TESTS (RAST)

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ABSTRACT

The diagnosis of an allergy in a patient can be very complicated. Over the years a number of methods have been developed for the detection of the small amounts of specific-IgE (sIgE) in human serum. Selecting the appropriate allergy test can be difficult and it should be in context of a patient's clinical presentation. The relevance of a test may also vary according to the patient's age, allergen exposure, and performance characteristics of the selected test method. It is important to note that positive sIgE results only indicate sensitisation, and are not diagnostic of the allergy without the appropriate exposure history. Large panels of indiscriminately performed allergy tests may therefore, provide misleading information if not interpreted correctly. Cross-reacting proteins/allergens, that may or may not have clinical relevance to allergic disease, may also influence tests for sIgE. It is thus clear that several factors should be taken into consideration when selecting and interpreting a sIgE allergy test.

INTRODUCTION

"Allergy" or allergic symptoms are common complaints from patients. The described symptoms and signs are also sometimes vague and very non-specific. The diagnosis of an allergy is very complicated and involves careful consideration. Immunoassays for allergen sIgE are often used to confirm the suspected clinical diagnosis of allergic disease. They are, however, often interpreted as diagnostic tests, which they hardly are. The interpretation of allergy tests is influenced by many factors, including the prevalence of the disease in the population being tested, and the sensitivity and specificity of the specific test.[1]

The sIgE test is a relatively new test in laboratory medicine, as the IgE antibody was only discovered in the late 1960's. The initial laboratory assays for quantifying serum sIgE used a radioisotope for labelling antibodies in an immunoassay. Figure 1 shows a schematic representation of the immunoassay method. This methodology, known as Radio-Allergosorbent Test or RAST, has since been replaced with newer technology where enzyme labelled indicators are used as markers in the assays. Although it is not precisely correct, the term 'RAST' is still used to refer to allergen sIgE tests.

The biochemical techniques used in immuneassays to analyse IgE antibodies, require considerable expertise as it is complicated by several factors. These factors include:

- Low IgE concentrations in blood. The concentration of IgE antibodies in the blood is considerably lower (10^{-9} times lower – ng/L vs g/L) than other antibodies in the serum. IgE antibodies are generally measured in IU/mL or kIU/L (one SI unit equals 1µg/L of protein).[2]
- Multiple antigen epitopes. Each allergen-protein contains multiple allergenic components or epitopes that IgE antibodies may recognise. Some patients may form antibodies to only some of the epitopes in a specific allergen. Some of the epitopes may be altered or destroyed in the processing of the immunoassay, therefore recombinant allergenic proteins have been introduced to the *in vitro* immunoassay system.[3]
- Reproducibility. The allergen and anti-IgE antibody used in the assay should be standardised to ensure reproducibility between batches and methods.[3]

Given the abovementioned concerns, the sIgE immuneassay should be i) sensitive to capture low concentrations of antibodies, and ii) use allergens from standardised sources that contain all of the allergenic epitopes related to the specific allergen.

Although the allergen specific-IgE test is commonly used by clinicians, it has specific limitations that might diminish its value.[3] These limitations include:

- Cross-reactivity between different allergen sources, which could cause clinically irrelevant positive test results. Some allergens may contain not only proteins, but also carbohydrate epitopes which may be recognised by an antibody. Since the structure of the carbohydrates may share similar homologies between allergen families, they are prone to extensive cross-re-

activity. Some proteins with similar structures between allergen families may also cause cross-reactivity. Although this cross-reactivity occurs *in vitro*, it is not always the case *in vivo*.[3,4]

- Negative and false negative results may occur, as not all allergens and allergen components have yet been characterised, and some allergens' allergenicity may be altered during reagent preparation.
- A positive sIgE test result only indicates sensitisation to an allergen, and not allergy.

INTERPRETATION OF sIgE RESULTS

Ideally, a diagnostic laboratory test for a specific diagnosis should yield either a positive or a negative result, with a 100% sensitivity and specificity. Unfortunately, despite the development of current generation immuneassays for IgE, the role of laboratory tests in the diagnosis of allergic diseases remains limited. It serves mainly to confirm a strongly suspected clinical diagnosis, based on a patient's extensive medical history and a thorough physical exam. When using published diagnostic 'decision points' for sIgE tests, it is important to take the following factors into consideration:

1. THE PREVALENCE OF A SPECIFIC ALLERGIC DISEASE IN THE POPULATION BEING TESTED

The predictive value of an allergy test is the probability that a subject with a positive test has an allergic condition, or the probability that a subject with a negative test does not have the allergy (refer to Tables I and II).[1,5] The predictive value of a test is influenced by the prevalence of the disease investigated in the specific population.

A value of 90% is generally acknowledged as the statistical cut-off point for accepted sensitivity and specificity. In order to explain the influence of the prevalence of a disease on the IgE allergy test, the following hypothetical examples will be used:

- **Example 1:** A "healthy population" with a prevalence of allergic disease of **20%** (Table III). If the population size is 100 individuals, of which 20 are clinically diagnosed and confirmed with atopy, then the true positive (TP) test results in this specific population will be 20 x sensitivity of test = 20 x 0.90 = 18. Thus 18 out of 20 atopic patients will be correctly identified with the specific allergy test. On the other hand, 80 individuals in this population do not have any clinical allergic condition, and the true negative (TN) test results can be calculated as 80 x specificity of test = 80 x 0.90 = 72. Thus 8 out of 80 healthy patients will be incorrectly classified as "allergic". In other words, the positive predictive value (PPV) of the test will be 69% and the negative predictive value (NPV) 97%. It follows that the probability that a subject with a "positive test" will have a clinical allergy is only 69%, but the probability that a subject with a "negative test" will not have an allergy is 97%. In this scenario the test application will be excellent for **excluding allergic conditions**. Reacting to the positive results, without taking anything else into consideration, may lead to unnecessary further investigations and dietary restrictions, for example, on the patient.
- **Example 2:** An "asthmatic population" with a prevalence

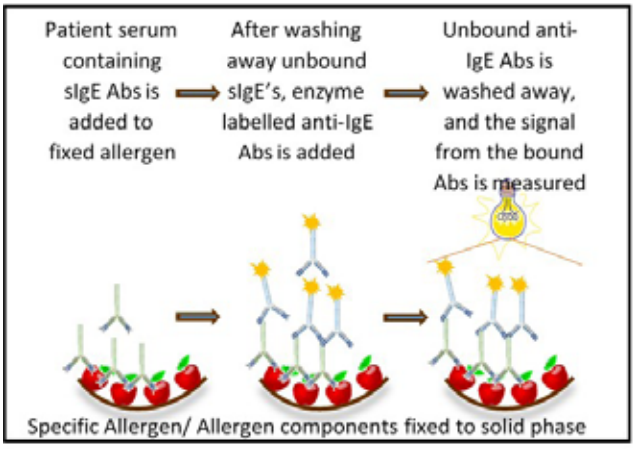

Figure 1: Schematic representation of the allergen specific-IgE immuneassay principle. The specific allergen/allergen-component is covalently bound to a solid phase. The patient's serum containing IgE antibodies is added, and the allergen-specific antibodies recognise and bind the allergen. The non-bound IgE antibodies are washed away, and anti-IgE antibodies, labelled with radioactive or enzyme-colorimetric marker, is added. The signal measured is directly proportional to the amount of allergen-specific antibodies in the patient's serum. Abbreviation: Abs = antibodies.[2]

TABLE I: DEFINITIONS AND ABBREVIATIONS OF SENSITIVITY, SPECIFICITY, PREDICTIVE VALUES AND PREVALENCE

POSITIVE PREDICTIVE VALUE (PPV)	Proportion of true positive test results among all positive test results.
NEGATIVE PREDICTIVE VALUE (NPV)	Proportion of true negative results among all negative test results.
SENSITIVITY (SENS)	Proportion of positive results among patients with the disease.
SPECIFICITY (SPEC)	Proportion of negative results among healthy patients.
PREVALENCE (PREV)	The total number of existing cases in a population.
TRUE POSITIVE (TP)	Number of sick patients with positive test results.
TRUE NEGATIVE (TN)	Number of healthy patients with negative test results.
FALSE POSITIVE (FP)	Number of healthy patients with positive test results.
FALSE NEGATIVE (FN)	Number of sick patients with negative test results.

TABLE II: CLASSIFICATION OF POSITIVE AND NEGATIVE TESTS RESULTS USING DEFINITIONS OF SENSITIVITY, SPECIFICITY, POSITIVE- AND NEGATIVE PREDICTIVE VALUES

CLASSIFICATION	POSITIVE TEST RESULT	NEGATIVE TEST RESULT	TOTAL NR	TEST DIAGNOSTIC INFORMATION
PATIENTS ***WITH* ALLERGY**	TP True Positive	FN False Negative	TP+FN (PREVALENCE)	Sensitivity % $= \frac{TP}{TP+FN} \times 100$
PATIENTS ***WITHOUT* ALLERGY**	FP False Positive	TN True Negative	FP+TN	Specificity % $= \frac{TN}{FP+TN} \times 100$
TOTAL NR PATIENTS	TP+FP	TN+FN	TP+TN+FP+FN (TOTAL POPULATION)	
PREDICTIVE VALUE	**PPV %** $= \frac{TP}{TP+FP} \times 100$	**NPV %** $= \frac{TN}{TN+FN} \times 100$	*TP is influenced by both sensitivity of the test and the prevalence of the disease:* *TP = (Sens%/100) x Prev*	

TABLE III: CLASSIFICATION OF POSITIVE AND NEGATIVE TESTS RESULTS IN A HEALTHY POPULATION (N = 100) WITH AN ALLERGY PREVALENCE OF 20%, USING AN ALLERGY TEST WITH 90% SENSITIVITY AND 90% SPECIFICITY

CLASSIFICATION	POSITIVE TEST RESULT	NEGATIVE TEST RESULT	TOTAL NR	TEST DIAGNOSTIC INFORMATION
PATIENTS ***WITH* ALLERGY**	20 x 0.90 = **18** TP	**2** FN	**20** Prevalence	**SENSITIVITY %** = 90 %
PATIENTS ***WITHOUT* ALLERGY**	**8** FP	80 x 0.90 = **72** TN	**80**	**SPECIFICITY %** = 90 %
TOTAL NR PATIENTS	26	74	100	
PREDICTIVE VALUE	**PPV %** 69 %	**NPV %** 97 %	*The low prevalence of the condition in the population renders the test an excellent tool to **EXCLUDE allergy**, with a **NPV of 97%***	

TABLE IV: CLASSIFICATION OF POSITIVE AND NEGATIVE TESTS RESULTS IN AN ASTHMATIC POPULATION (N = 100) WITH AN ALLERGY PREVALENCE OF 70%, USING AN ALLERGY TEST WITH 90% SENSITIVITY AND 90% SPECIFICITY

CLASSIFICATION	POSITIVE TEST RESULT	NEGATIVE TEST RESULT	TOTAL NR	TEST DIAGNOSTIC INFORMATION
PATIENTS ***WITH*** ALLERGY	70 x 0.90 = **63** TP	**7** FN	**70** Prevalence	**SENSITIVITY %** = 90 %
PATIENTS ***WITHOUT*** ALLERGY	**3** FP	30 x 0.90 = **27** TN	**30**	**SPECIFICITY %** = 90 %
TOTAL NR PATIENTS	66	34	100	
PREDICTIVE VALUE	**PPV %** = 96 %	**NPV %** **= 79 %**	*The high prevalence of allergic conditions in the population renders the test an excellent tool to **SCREEN for allergy**, with a **PPV of 96%***	

of allergic disease of **70%** (Table IV). If the population size is 100 individuals, of which 70 are clinically diagnosed and confirmed with atopy, then the TP test results in this specific population will be 70 x sensitivity of the test = 63. This means that 63 out of 70 patients with atopy will be correctly identified as "allergic" by the test, and 7 patients will be "missed". On the other hand, 30 individuals in this population do not have any clinical allergic condition, and the TN test results can be calculated as 30 x spec = 30 x 0.90 = 27. Thus 27 out of 30 patients will be correctly identified as healthy, and 3 will be misclassified as "allergic". The PPV of the test compared with the first population group will increase to 96% and the NPV will decrease to 79%. In this scenario this test may be best applied as a **screening test** for atopy.

When interpreting sIgE test results it is important to consider the prevalence of allergy in the patient population being tested. The higher the prevalence of allergic disease in the patient population group, the higher the predictive value of a positive allergy test.[1,5] It is important not to be misled by the published sensitivity and specificity of a specific test alone.

2. TEST METHOD

The immuneassay test methods used by the manufacturers of these tests are not always the same. For some sIgE allergens it is particularly important to consider the manufacturer's specific test method, as many factors may influence it. The production quality of the allergen and the anti-IgE antibodies may differ between manufacturers.[4] Reported sensitivities and specificities may also only apply to a specific

TABLE V: GENERIC REFERENCE RANGES FOR sIgE (IMMUNOCAP®, THERMOFISHER)			
RANGE (KU/L)		DESCRIPTION	INTERPRETATION AND MANAGEMENT OPTIONS
< 0.10		Undetectable	Consider causes other than IgE-mediated allergy to explain the symptoms
0.10 - 0.35		Detectable, low	In rare cases patients with antibody levels in this range may experience clinical symptoms. Correlate with clinical findings.
0.36 - 0.69	1	Low	Increased sIgE to an allergen only indicates sensitisation. A diagnosis of IgE-mediated allergy requires evidence of both sensitisation and clinical reactivity. In these cases consider: • Allergen avoidance; • Desensitisation; • Symptomatic treatment.
0.70 - 3.49	2	Moderate	
3.50 - 17.40	3	High	
17.50 - 49.0	4	Very high	
- 99.0	5		
> 100.00	6		
ALL sIgE TEST RESULTS SHOULD ALWAYS BE CORRELATED WITH THE CLINICAL HISTORY			

test method, as may the detection limits and cut-off values between manufacturers. It is very important that laboratories state the specific method in use on the laboratory report, as there is no standardisation of quantitative results between different test methods. Most published data on immuneassays used in the diagnosis of allergic conditions, are based on the ImmunoCAP® assay (ThermoFisher, Sweden), previously called the RAST test. This is also the most utilised allergy test system in South Africa by the larger laboratories.

3. OTHER FACTORS

The likelihood of clinical reactivity is influenced by the patient's specific clinical history, degree of positivity and the specific allergen in question. The higher the concentration of antibodies in the patient, the more likely the patient is to experience symptoms upon exposure to the allergen (Figure 2).[1,3,6] However, low concentrations of antibodies to a specific allergen still denote a certain degree of probability for a clinical reaction,[3] which is the case in especially drugs, venoms and nuts. The exact relationship between sIgE and disease activity is not clearly understood, and further studies are needed to investigate this relationship.

REPORTING OF ALLERGEN sIgE RESULTS

Due to the above discussions, qualitatively reporting an allergy immuneassay test result as positive or negative, is not always diagnostically appropriate. Quantitative sIgE results are reported in quantitative units namely kIU/L. As stated, the most published data on immuneassays used in the diagnosis of allergic conditions are based on the ImmunoCAP® assay from ThermoFisher in Sweden. The ImmunoCAP® assay's lower limit of detection is 0.10 kU/L, therefore undetectable sIgE concentrations are reported as less than 0.10 kIU/L. Previously the lower limit of detection on the older immuneassays was 0.35 kIU/L, and older studies will quote a positive sIgE as > 0.35 kU/L. Detectable allergen levels, even if the concentration is below 0.35 kIU/L, should always be correlated with clinical symptoms, as some allergens may cause clinical symptoms even at low concentrations.

For most allergens, the likelihood of clinical symptoms increase at higher concentrations of sIgE (Table V). For this fact diagnostic "cut-off points" have been proposed in a number of studies with a 95% PPV for the major food allergens.[5,6] These studies also discriminate between age groups for certain allergen sIgE, e.g. egg and cow's milk proteins. It is important to note that the studies were performed on the ImmunoCAP® sIgE method, and that different reference ranges and predictive values may apply to different sIgE methods.

CONCLUSION

Immunoassays for allergen sIgE should only be used to confirm the suspected clinical diagnosis of allergic disease and should not be readily regarded as diagnostic tests. The selection of allergy diagnostic tests and interpretation of allergen sIgE antibody results MUST always be guided and viewed within the context of the patient's clinical history, regardless of reported diagnostic cut-off points. The specific immuneassay method, as well as possible interferences and shortcomings of the specific assay, should always be taken into consideration when selecting and interpreting a sIgE test result.

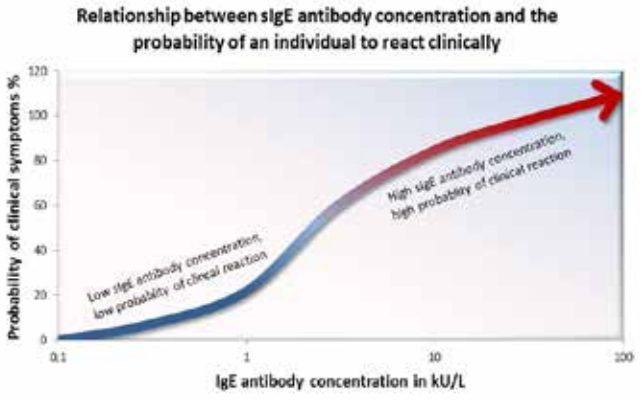

Figure 2: The relationship between sIgE antibody and the probability of a clinical reaction. The higher the concentration of sIgE, the higher the probability to be allergic to the specific allergen. The diagnosis of allergy remains mainly dependent on the clinical history, limiting the diagnostic role of the laboratory test.

DECLARATION OF CONFLICT OF INTEREST

The author declares no conflict of interest.

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