

ATTACHMENT-26

Radioallergosorbent Test (RAST) Methods for Allergen-Specific Immunoglobulin E (IgE) 510(k)s; Final Guidance for Industry and FDA

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**This document supersedes Review Criteria For Assessment Of Allergen-Specific
Immunoglobulin E (IgE) *In vitro* Diagnostic Devices Using Immunological Test
Methodologies, March 2, 1993**



**U.S. Department Of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health**

**Immunology Branch
Division of Clinical Laboratory Devices
Office of Device Evaluation**

Preface

Public Comment

Comments and suggestions may be submitted at any time for Agency consideration to Dockets Management Branch, Division of Management Systems and Policy, Office of Human Resources and Management Services, Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD, 20852. When submitting comments, please refer to the exact title of this guidance document. Comments may not be acted upon by the Agency until the document is next revised or updated.

For questions regarding the use or interpretation of this guidance contact Geretta P. Wood at (301) 594-1293 or email gpw@cdrh.fda.gov.

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Radioallergosorbent Test (RAST) Methods for Allergen-Specific Immunoglobulin E (IgE) 510(k)s; Final Guidance for Industry and FDA

This document is intended to provide guidance. It represents the Agency's current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind Food and Drug Administration (FDA) or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute and regulations.

PURPOSE OF THE GUIDANCE

This document describes FDA's current recommendations for the kind of data and information you should provide in premarket notifications (510(k)s) for Radioallergosorbent Tests (RAST) Methods for Allergen-Specific Immunoglobulin E (IgE) test systems. RAST testing measures specific allergen antibodies and may aid in the diagnosis of asthma, allergies, and other pulmonary disorders. This document has been modified to address those allergens for which few patient samples are available (rare allergens).

THE LEAST BURDENSOME APPROACH

The issues identified in this guidance document represent those that we believe need to be addressed before your device can be approved or cleared for marketing. In developing the guidance, we carefully considered the relevant statutory criteria for Agency decision-making. We also considered the burden that may be incurred in your attempt to comply with the guidance and address the issues we have identified. We believe that we have considered the least burdensome approach to resolving the issues presented in the guidance document. If, however, you believe that information is being requested that is not relevant to the regulatory decision for your pending application or that there is a less burdensome way to address the issues, you should follow the procedures outlined in the "A Suggested Approach to Resolving Least Burdensome Issues" document. It is available on our Center webpage at:
<http://www.fda.gov/cdrh/modact/leastburdensome.html>

IDENTIFICATION OF ALLERGEN-SPECIFIC IMMUNOGLOBULIN E (IgE) IMMUNOLOGICAL TEST SYSTEMS AND THEIR CLINICAL INTENDED USES

This generic type of device is intended for use in clinical laboratories and physicians' office laboratories as an *in vitro* diagnostic test for quantitative, semi-quantitative or qualitative measurement of allergen-specific IgE by various immunological test methodologies such as radioimmunoassay, enzyme immunoassay, chemiluminescent immunoassay, and fluorescent immunoassay.

PRODUCT CODE: 82 DHB, RADIOALLERGOSORBENT (RAST) IMMUNOLOGICAL TEST SYSTEM

REGULATION: 21 CFR §866.5750

- (a) Identification. A radioallergosorbent immunological test system is a device that consists of the reagents used to measure by immunochemical techniques the allergen antibodies (antibodies which cause an allergic reaction) specific for a given allergen. Measurement of specific allergen antibodies may aid in diagnosis of asthma, allergies and other pulmonary disorders.
- (b) Classification. Class II (performance characteristics)

PANEL: 82: Immunology Devices Panel

PREDICATE DEVICE: Acceptable predicate devices are Radioallergosorbent (RAST) Immunological Test Systems that have been cleared by FDA via 510(k) notification for commercial marketing.

I. INTRODUCTION

Allergic or immediate hypersensitivity reactions are known as Type I immunopathologic reactions that may occur within minutes of an allergic challenge. In 1967 Ishizaka identified the serum factor capable of mediating allergic reactivity as Immunoglobulin E (IgE).¹ The Fc portion of this reaginic antibody attaches to Fc receptors on the surface of target cells, tissue mast cells, and circulating basophils leaving the F(ab)₂ portion of the molecule available to bind with its homologous antigen. Upon contact with a specific allergen, the IgE mediates cell release of pharmacologic substances such as histamine, prostaglandins, and leukotrienes that result in allergic reactions ranging from hay fever, urticaria (hives), and bronchial asthma, to generalized anaphylactic shock.^{1,2}

The discovery of the role of IgE in clinical allergy resulted in a new generation of *in vitro* diagnostic assays to test for allergen sensitivity. The first immunoassays were developed to quantitate the serum concentration of total IgE. In normal individuals, IgE is usually present at low levels where 130 ng/mL represents the upper limit of the normal range. However, a significant number of asymptomatic normal individuals, patients with parasitic diseases and patients with depressed cell-mediated immunity exceed this level.

Also, some allergic (atopic) persons may exhibit normal total IgE test results in the presence of elevated levels of specific IgE. Therefore, although the total serum IgE level is considered useful in the evaluation of an allergic patient, more important is the demonstration of allergen-specific IgE in a patient's serum. RAST was developed for this purpose.

Historically, the diagnosis of allergic disease has been based primarily on two types of *in vivo* testing: provocation (challenge testing) and skin testing. The use of provocation testing involves masked (double-blind) challenge that duplicates natural exposure.¹⁶ Responses to challenge other than by natural exposure are dependent on physical characteristics of test material as well as on the dose and duration of exposure to it.¹⁶ Skin testing, either intradermal or prick/puncture (epicutaneous), is a frequently used clinical method of diagnosing allergy. Although these tests do produce false results in some instances, for certain allergens they exhibit greater clinical sensitivity than RAST.¹⁷ However, other recognized clinical modalities may also be used to assess the clinical status of patients. These include nasal or bronchial challenge tests for diagnosing inhalant allergies, and oral challenge testing for diagnosis of food allergies. *In vitro* allergen-specific IgE testing is especially useful when skin testing cannot be performed or interpreted in patients with generalized dermatitis, or in those who must continue to take antihistamine medications.¹⁶ *In vitro* testing also eliminates the risk of possible systemic reaction.¹⁸ It is essential that allergy test results, regardless of the procedure employed, reflect true clinical sensitivity and specificity in order to prevent mismanagement of patients.

The best correlation between RAST-type tests and skin tests have been with inhalant allergens such as the pollens of trees and grasses. FDA has noted in data received from various sponsors that concordance with skin tests varies with different allergens and can range from greater than 80% for some allergens to less than 20% for other allergens. Therefore, FDA requests manufacturers to provide allergy specific clinical data for each new allergen they submit for their RAST-type test.

Atopic status of patients may also be defined by physical examination and clinical history and should be included as part of the comparison data. Since January 1990, package inserts have included the concordance for each allergen between the RAST-type test and the comparative clinical test if the concordance is below 65%.

In order to alert RAST users to an important potential cause of false negative results for food allergies, the following statement should be incorporated into the Limitations section of the package insert.

When testing for food allergies, circulating IgE antibodies may not be detected if they are directed towards altered forms of allergens (such as cooked, processed or digested) and the altered forms are not present in the same form as those food allergens which are used in the test.

Two other tests for food allergies that are the subjects of scientific concern are cytotoxicity testing, and the measurement of serum levels of IgG and IgG4 antibodies to specific food allergens. Clinical data for these two tests are not conclusive for diagnosis of food allergies. This document addresses allergen-specific IgE only and does not include IgG sensitivities for food allergies. A specific set of clinical data would be needed to support the safety and effectiveness for measurement of IgG antibodies to specific food allergens.

II. DEVICE DESCRIPTION

A. Intended Use:

A radioallergosorbent immunological test system is a device that consists of the reagents used to measure by immunochemical techniques the antibodies that cause an allergic reaction specific for a given allergen. Measurement of allergen specific antibodies may aid in the diagnosis of asthma, allergies, and other pulmonary disorders.

B. Detailed Principle of Test Methodology:

RAST Immunological Test System devices use the following general principles that vary according to methodology employed. A specific allergen is bound to a solid-phase support with a microtiter well, glass or magnetized beads, or some other inert surface. Patient serum containing IgE, both specific and not specific for the testing allergen, is incubated with the solid phase material, allowing reaction of the specific IgE in the patient sample. Excess serum and non-allergen specific IgE are then washed away. Labeled anti-IgE antibody conjugate is added. During this 2nd incubation period a sandwich complex of allergen-patient IgE allergen specific antibody-labeled anti-IgE is formed. A subsequent wash removes unbound labeled antibody. Measurement of the remaining labeled anti-IgE is directly proportional to the patient's allergen specific IgE. Examples of indicator systems (labels) used in RAST are ^{125}I for radioimmunoassay (RIA) systems and alkaline phosphatase, horse radish peroxidase or urease for enzyme immunoassay (EIA) systems.

Include the following items for each 510(k):

1. Each specific allergen contained in the submission should be individually listed.
2. Include a statement on whether the test is qualitative, semi-quantitative, or quantitative. Although some commercial RAST-type kits provide test results by a qualitative procedure, most of these devices are designated as semi-quantitative due to the arbitrary units of measurement assigned to each test method. Quantitative tests are traceable to a World Health Organization (WHO) IgE Standard.

3. The specific IgE units of measurement are interpreted by a scoring system (allergy classification), with classes ranging from 0 to 6. Scoring systems consisting of arbitrary units will vary according to individual test systems. Subsequent procedural laboratory instructions will vary with the test method that is utilized. Classification interpretation and procedural instructions should be fully described in the package insert.

An example of establishing a RAST scoring system is by use of reference points generated by assaying four to five dilutions of a pooled human reference serum containing IgE antibodies specific for a single allergen. Arbitrary units and RAST classes are assigned to each reference point. Respective RAST classes are assigned to the patient serum tested based on the relationship of the test serum to the reference serum points.²

4. Antisera and conjugates used in RAST assays should be fully described, with identification of antibody source, and whether it is polyclonal or monoclonal. Identify any buffers, stabilizers, and preservatives contained in the reagent.

Examples of antisera commonly used are rabbit IgG, anti-human IgE or mouse monoclonal anti-human IgE that is labeled with radioactive iodine (¹²⁵I), or an enzyme such as horseradish peroxidase, alkaline phosphatase, or urease.

5. Substrate or indicator reagents used in EIA kits should be described, including pH values. Examples include, but are not limited to, Bromocresol Purple indicator and urea substrate (pH 4.8), para-nitrophenyl phosphate (PNP), and fluorogenic substrates. Preservatives contained in these reagents should also be identified, e.g. sodium azide, thimerosal, etc. A complete description of the enzyme-substrate reaction and interpretation should be included. Substrates will vary for each test kit relative to the appropriateness of the chemical reaction with the enzyme label.
6. Quality control reagents (calibrators, controls) should be identified and instructions for use should be included in a separate section of the labeling.
 - a. Positive and negative controls should be included in every run to verify assay performance. Mean values and acceptable ranges of the controls should be determined by replicate assays.
 - b. IgE calibrators (reference sera) should be traceable to a recognized reference preparation such as the World Health Organization or 1st British Standard for Human Serum IgE. All calibrators and control sera should be run in duplicate in order to minimize random errors.

III. CLINICAL AND NONCLINICAL LABORATORY STUDIES: SPECIFIC PERFORMANCE CHARACTERISTICS

FDA requests different types and amounts of data and statistical analyses in applications to market *in vitro* diagnostic devices depending upon the following criteria:

- analyte
- intended use (which determines whether the application is a 510 (k))
- technological characteristics

Data and statistical evaluation should be sufficient to determine if the device is substantially equivalent to a legally marketed device. Manufacturers of RAST and RAST-type test kits should also submit clinical data for each specific allergen that is to be used with the test system. Data for each allergen should include a parallel comparison between RAST results and corresponding individual allergen-specific clinical information in order to demonstrate correlation with the clinical diagnosis of allergy patients. Clinical symptoms manifested in the population tested (i.e., atopic patients with seasonal allergy symptoms, allergic rhinitis, etc.) should be defined.

If monoclonal IgE antibodies are used in the assay, a summary of characterization should be submitted, including the following:

- identification of source of parent myeloma cell
- source of antibody (mouse, etc.)
- antibody characterization
- description of cloning and criteria used for antibody selection
- stability data (summary of real-time studies)
- precision, accuracy, reproducibility, recovery and linearity data
- summary of data demonstrating lot-to-lot consistency for 3 lots

FDA recommends that you provide the following analytical and clinical studies in support of substantial equivalence.

A. Analytical Studies

1. Method Comparison

- a. Show that the performance of the device is substantially equivalent to another legally marketed commercial product available in the United States and, if applicable, when compared to a reference method. For quantitative or semi-quantitative tests, compare results using RAST samples free from interfering substances from 50 to 100 persons covering the entire working range of the assay (from low to high levels).⁷ Analyze the data using linear regression or other acceptable methods.⁷ For rare allergens, additional analytical studies may be

necessary to compensate for the small number of clinical samples available. This may include inhibition studies performed on positive patient samples, antibody detection in animal models, etc.

- b. Present test data with estimates of error or confidence intervals, if appropriate, and analyses and conclusions.

2. Specimen Matrices

- a. Include data to support use of the test with all specimen matrices claimed in the intended use.

3. Precision^{3,6}

- a. For quantitative or semi-quantitative tests, calculate total, between and within day, between and within-run means, and coefficients of variation of imprecision.
- b. Report in the performance characteristics section of the package insert, the appropriate means, SDs and coefficients of variation with confidence levels according to number of replicates assayed. Report the number of runs per day.

4. Minimal Detectable Quantity

- a. The analytical sensitivity is defined as the lowest quantity differentiated from Zero (95% confidence intervals or 2 standard deviations are commonly used).³
- b. Run the Zero standard (diluent) at least 20 times in the same run and calculate the mean of the Zero standard and two standard deviations (SDI) of the mean.

B. Clinical Studies

1. Allergen-Specific Performance Characteristics

Describe all protocols for studies. Present test data with concordance or correlation values, estimated parameters and confidence intervals, analyses for inference, and conclusions.

- a. Provide correlation with clinical parameters such as patient history or *in vivo* test results performed according to standard medical procedure.

Compare random population clinical samples ($n = 35$ to 50) using specific allergen discs, with corresponding clinical parameters which

are used for comparison, and another commercial (similar) test system.

Identify criteria used for patient selection (according to clinical allergy history and physical examination). Present the above data in tabular form.

- b. Provide clinical specificity data; test non-atopic patients (n = 35 to 50). Include statement of criteria used for patient selection, according to clinical allergy history and physical examination. Provide calculation of results as follows:

$$\text{Clinical Specificity} = \frac{\text{\# non-atopic samples with a negative test (TN)}}{\text{Total \# non-atopic samples (FP + TN)}} \times 100$$

- c. Provide clinical sensitivity data; test atopic patients (n = 35 to 50). Include statement of criteria used for patient selection according to clinical allergy history, and physical examination. Provide calculation of results as follows:

$$\text{Clinical Specificity} = \frac{\text{\# non-atopic samples with a positive test (TP)}}{\text{Total \# atopic samples (TP + FN)}} \times 100$$

Results may be presented in tabular form as below:

	# positive	# negative	Totals
Atopic patients	TP	FN	TP + FN
Non-atopic patients	FP	TN	FP + TN
Totals	TP + FP	FN + TN	TP + FP + TN + FN

2. Physician's Office Laboratories (POLs)

All test protocols for RAST *in vitro* devices intended for point-of-care should be clearly stated with directions and acceptance criteria for selecting clinical patients for inclusion in the study population. Subjects should sign patient consent forms. The duration of the clinical study should be stated. The amount and types of data requested depend on the intended use and the technological characteristics of the new device. Include the following information:

- a. data from three laboratory testing sites representing variety in sizes of the POLs

- b. the amount and types of laboratory training of the personnel who perform the tests (personnel with a variety of training should be represented)
- c. laboratory identification with the corresponding patient data

3. Automated Instrumentation

Some RAST devices are dedicated for use on automated analyzers where software elements and microprocessor-controlled devices are an integral part of the test system.

You should review the recommendations for moderate level of concern devices given in **Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices** issued on May 29, 1998. It is available on the Internet at <http://www.fda.gov/cdrh/ode/57.html>

4. Identification of Allergenic Extracts

The following information and laboratory studies should be included in a submission for each specific allergen intended for use in RAST Immunological Test Systems. All respective studies should have a summary of conclusions.

- a. Source of extracts
 - 1) Specific allergen extracts should be purchased from a laboratory licensed by the FDA Center for Biologics Evaluation and Research (CBER).
 - 2) If not purchased from, or produced by a licensed laboratory, the company should maintain characterization data on file, and provide a summary statement of preparation or purification (in accordance with CBER protocols). For extracts that have been standardized by CBER, device manufacturers should use those extracts, or demonstrate equivalence of their extracts with standardized extracts.
- b. In order to achieve the maximum binding of patient's specific IgE antibodies, manufacturers should set specifications (derived from testing) for the highest concentration of extract bound to a solid support in a quantity that will produce maximum saturation.
- c. Specificity of the allergen should be demonstrated by evaluation of RAST inhibition testing.

- d. Cross-reactivity with similar antigens may be substantiated by provision of literature references regarding such cross-reactivity, or provision of any data obtained by testing.
- e. Test the device as appropriate for the following possible cross-reactive or interfering substances: Immunoglobulins G,A,M; Human Serum Albumin; endogenous substances at high concentrations such as lipids, hemoglobin, bilirubin, etc.^{3,5}; and drugs or medications commonly used by patients who are tested for total or allergen-specific IgE.
- f. If extracts contain a mixture of allergens, the manufacturer should provide the rationale for grouping. Data should be provided to demonstrate comparison of test results between the mixed extract and individual components of that mix.
- g. A summary of lot-to-lot reproducibility data of 3 lots of specific allergens should be provided for each allergen. The manufacturer should keep a reference serum pool from untreated patients with each specific allergy.
- h. If more than one RAST test system using different methodology is manufactured by a company, correlation between each method should be demonstrated for each allergen (n = 35 to 100, with flexibility for rare allergens).
- i. If allergen discs and other components of the test system are to be marketed for use with other manufacturers' test systems, the manufacturers of those discs and components should provide comparison data derived from those test systems. In addition, labeling for those allergens should identify the tests systems evaluated.
- j. Real-time stability studies for each allergen should be available to substantiate expiration dating.

5. IgE Reference Material

IgE standards used as reference material for RAST assays should be traceable to the Second International Reference Preparation (IRP) 75/502, Human Serum Immunoglobulin E from the World Health Organization, the United States IgE Standard, or another recognized IgE reference preparation.

6. Allergen Specific IgE Control Sera

Allergen specific control sera may be marketed as a component of a complete RAST kit, or as a separate device. If it is to be marketed as a

separate device, a 510(k) notification is required which should contain the following information:

a. **Intended use statement**

A statement should be provided to indicate if the control is designated for use with a specific test system only, or if it may be used with multiple assay systems.

b. **Labeling**

1) If the control serum is dedicated to a specific test system, the following data should be provided in the package insert: target range values, means, standard deviations, and number of replicates used to establish those values. If the control serum is intended for use with multiple assay systems, those test systems should be individually listed in the labeling of the control kit, with the above data obtained from each of the test systems.

2) If the control serum is to be used with multiple assays, comparative data with a predicate control kit should be submitted.

c. **Source**

Identification of the source of the allergen-specific IgE contained in the control serum should be provided.

d. **Stability**

Data obtained from real-time stability studies should be available to support expiration dating of the control serum.

e. **Specificity**

Method of confirmation of allergen specific IgE specificity should be identified for each specific allergen identified in the control. A summary of data obtained by testing should be provided.

f. **Mixed Allergen Controls**

Method of confirmation and data for individual allergen specificity should be provided for each specific allergen identified in the mix.

IV. LABELING

The following are additional details for some of the points given above or in the *in vitro* diagnostic device labeling regulations, 21 CFR 809.10(b).

A. The Intended Use Statement and Indications for Use

The intended use statement should be a concise description of the essential information about the device and should communicate the following information.

1. Identification of the analyte and each specific allergen that is included in the submission
2. Whether the assay is quantitative, semi-quantitative or qualitative
3. Test methodology
4. If the test is to be used only with a specific instrument
5. Special instrumentation requirements
6. Specify test matrix, i.e., human serum or plasma
7. Sites for use, i.e., in clinical laboratories or point-of-care
8. Diagnostic application

Provide a statement that the device is intended to be used as an aid in the clinical diagnosis of IgE mediated (Type I) allergy in conjunction with other clinical findings.

9. Clinical Significance

If the clinical significance can be stated in a few words it may be included in the intended use section. If the clinical significance statement is lengthy or complicated, create a separate heading entitled Clinical Significance.

B. Quality Control

You should include the following information.¹⁰

1. Controls

Specimens or commercially available products that should be used for positive and negative control including recommended levels of analyte, if materials are not provided in the kit.

2. Recommendations for quality control parameters other than positive and negative controls, if appropriate.
3. Directions for performing quality control
4. Recommendations for frequency and placement of quality control samples within run and from run to run.
5. Directions for interpretation of the results of quality control samples (satisfactory limits of performance)
6. Conclude with a statement similar to the following: **If controls do not behave as expected, assay results are invalid.**

C. Limitations of the Procedure

You should list important test limitations and known contraindications, with references. Labeling for RAST tests should include the following limitations:

1. Definitive clinical diagnosis should not be based on the results of any single diagnostic test, but should be made by the physician after all clinical and laboratory findings such as *in vivo* skin tests or challenge tests, clinical history and results of physical examination are evaluated.
2. The results of an *in vitro* allergy test should not be the basis for selection of doses for immunotherapy.
3. Insect venom IgE - Patients who are allergic to insect venom may produce a variation in levels or small amounts of circulating allergen specific IgE in the range of class 0 or 1, while symptoms and possibility of current or future hypersensitivity are present.¹⁰
4. Food allergies - Circulating IgE antibodies may remain undetectable despite a positive clinical history. This is due to the fact that antibodies may be directed toward allergens that are revealed or altered during industrial processing, cooking, or digestion, and therefore do not exist in the original food for which the patient is tested. False positive test results in persons who are tested for food allergies may lead to inappropriate dietary restriction, while false negative results in food sensitive persons may result in anaphylactic reactions of varying severity.
5. Inhalant allergies - False positive results in persons who are tested for specific inhalant allergies may result in improper medication of those persons. False negative test results in such persons may result in lack of proper medical treatment.

6. When total IgE values are greater than or equal to 500 U/mL, low level allergen-specific IgE response should be interpreted with caution.
7. For more precise measurement of high-titered, allergen-specific IgE antibody levels, an appropriate recommendation for dilution of the patient sample, as well as calculation of results, should be included in this section of the labeling.
8. The binding capacity of specific IgE antibody may vary from allergen to allergen. Therefore, identical results for different allergens may not necessarily imply clinical equivalence.
9. Reliable and reproducible results will be obtained when the assay is performed according to the procedural instructions and with adherence to good laboratory practices.

D. Expected Values

Explain how to interpret test results:

1. Provide reference ranges for normal and abnormal patient populations.
2. Provide an explanation and illustration of the RAST scoring system and allergy classification to be used in the evaluation of test results. You should discuss how to interpret positive, negative, and equivocal or borderline results and describe their clinical significance.

E. Performance Characteristics

Supporting data - Summarize the data upon which the performance characteristics are based, e.g., accuracy, precision (repeatability and reproducibility), specificity, and sensitivity.

F. Labeling for Rare Allergens

The System Manual for RAST tests should include a discussion and listing of rare allergens along with details regarding the procedures utilized by the company to validate them and how that procedure is different from the validation for commonly occurring allergens. The manufacturer should devise a unique vial label for the rare allergens so that the user can readily differentiate those from the allergens validated in the usual fashion. The System Manual should indicate how the user can differentiate rare allergens, whether it is by a prefix or suffix to the product number or the color of the vial label.

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