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## Memorandum

Date SEP 1 1992  
From Chief, Immunology Branch, Division of Clinical Laboratory Devices, Office of Device Evaluation, Center for Devices and Radiological Health  
Subject Draft Guidance Document for 510(k) Submission of Immunoglobulins A,G,M,D and E Immunoglobulin System In Vitro Devices.  
To Interested Parties

We have developed a draft document entitled, "Review Criteria for Assessment of Immunoglobulin A,G,M,D and E Immunoglobulin System in Vitro Devices" that includes a generic model package insert. It is intended to assist manufacturers in the preparation of marketing submissions for these types of devices that we will be reviewing.

We are soliciting your ideas, recommendations, and comments regarding the enclosed draft guidance document. We will appreciate receiving your comments so we can incorporate as many improvements as possible in a revision. Additional copies of this document may be obtained through the Office of Small Manufacturers Assistance from Geoffrey Clark, (301) 443-6597.

Please address comments to:

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Attachment

Immunoglobulin Guidance: August 1992

Original: August 1992

**REVIEW CRITERIA FOR ASSESSMENT OF IMMUNOGLOBULINS A, G, M, D,  
AND E IMMUNOGLOBULIN SYSTEM IN VITRO DIAGNOSTIC DEVICES**

This is a flexible document representing the current major concerns and suggestions regarding immunoglobulins A, G, M, D, and E immunoglobulin system in vitro diagnostic devices. It is based on (1) current basic science, (2) clinical experience, (3) previous submissions by manufacturers to the FDA, and (4) the Safe Medical Devices Act of 1990 (SMDA) and FDA regulations in the Code of Federal Regulations (CFR). As advances are made in science and medicine and changes in implementation of Congressional legislation, these review criteria will be re-evaluated and revised as necessary to accommodate new knowledge.

**PURPOSE**

The purpose of this document is to provide guidance and clarification on information to present to the Food and Drug Administration (FDA) before a device to detect and quantitate Immunoglobulins A, G, M, D, and E in clinical specimens can be cleared for marketing.

A premarket notification [510(k)] submission must provide evidence that the device is accurate, safe, effective and substantially equivalent to a predicate device legally marketed in the United States.

**DEFINITION**

This generic type of device is intended for use in clinical laboratories and physician's offices\* as an in vitro diagnostic test for the semi-quantitative and quantitative measurement of immunoglobulins A, G, M, D, and E immunoglobulin system in vitro diagnostic devices using immunochemical and various other methodologies.

Devices may be cleared for use in physician's office laboratories when additional data is submitted to demonstrate equivalent performance in these settings

**PRODUCT CODES:**

IgA	82-CZP
IgG	82-DEW
IgG (Fab)	82-DFK
IgG (FC)	82-DAS
IgG (FD)	82-DAQ
IgG (gamma)	82-DFZ
IgD	82-CZJ
IgE	82-DGC
IgM (mu)	82-DAO
IgM	82-DFT

**REGULATION NUMBER:**

21 CFR § 866.5510, 866.5520, 866.5530, 866.5540, and 866.5550

- (a) **Identification.** Immunoglobulins A, G, M, D, and E immunological test system are devices that consist of the reagents used to measure by immunochemical techniques immunoglobulins A, G, M, D, and E (antibodies) in serum. Measurement of these immunoglobulins aid in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious agents.

Immunoglobulin G (Fab fragment specific) immunoglobulin test system is a device that consists of the reagents used to measure by immunochemical techniques the Fab antigen-binding fragment resulting from breakdown of immunoglobulin G antibodies. Measurement of Fab fragments of immunoglobulin G aids in the diagnosis of lymphoproliferative disorders, and Waldenstrom's macroglobulinemia.

Immunoglobulin G (Fc fragment specific) immunological test system is a device that consists of the reagents used to measure by immunochemical techniques the Fc (crystalline) fragment of immunoglobulin G antibodies. Measurement of immunoglobulin G Fc fragments aids in the diagnosis of plasma cell antibody-forming abnormalities.

(b) Classification. Class II (performance standards)

(c) Panel: Immunology

REVIEW REQUIRED:

I. CLINICAL INDICATION/SIGNIFICANCE/INTENDED USE OF  
IMMUNOGLOBULINS A, G, M, D, AND E IMMUNOLOGICAL  
ANALYTE DETECTION DEVICES

Immunoglobulins are a group of globular proteins sharing certain basic structural features, which can function as antibodies. Immunoglobulins are found in circulation, various secretions, and body fluids other than blood, as well as fixed on cell surfaces. Five major structural types or classes have been described in humans: IgG, IgA, IgM, IgD and IgE. Immunoglobulin diversity is controlled by immunoglobulin genes. The basic molecular subunit of the various immunoglobulin classes consists of four polypeptide chains of amino acids; two heavy chains and two light chains (1,2). The light and heavy chains are linked covalently by inter-chain disulfide (S-S) bonds. The light chains, designated kappa ( $\kappa$ ) or lambda ( $\lambda$ ), are shared by the five classes. An individual immunoglobulin molecule will contain either two kappa or two lambda light chains. The molecular basis for the differentiation of the five classes of immunoglobulins resides in the constant region of the heavy chain type; IgG, IgA, IgM, IgD and IgE heavy chains have been designated gamma ( $\gamma$ ), alpha ( $\alpha$ ), mu ( $\mu$ ), delta ( $\delta$ ), and epsilon ( $\epsilon$ ), respectively. Immunochemical and biochemical studies of myeloma proteins has led to the identification of subclasses for IgG, IgA and IgM. Four distinct subclasses of IgG have been described: IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, and IgG<sub>4</sub> (7,8) and two subclasses each for IgA and IgM; IgA<sub>1</sub>, IgA<sub>2</sub>, and IgM<sub>1</sub>, and IgM<sub>2</sub>. The basis for this subclass distinction resides in amino acid sequence variation in certain areas of the respective heavy chains. Recognition that IgG consists actually of four subclasses has provided knowledge regarding the biology of each subclass. Of particular interest has been the recognition that various antibodies are restricted to some subclasses. Immunoglobulin G subclass deficiencies are being increasingly recognized, especially in association with infection (4,5).

Three categories of immunoglobulin disturbances have been defined: 1) class-specific (monoclonal) increase of immunoglobulins (monoclonal gammopathies); 2) a decrease or absence of immunoglobulins (hypoimmunoglobulinemia), and 3) non class-specific (polyclonal) increase in immunoglobulins (hyperimmunoglobulinemia).

The association of monoclonal gammopathies with certain disease states has important diagnostic value and provides additional information with respect to disease activity. Monoclonal components represent a large amount of homogeneous protein (intact immunoglobulin, heavy, or light chains) produced by a single line or clone of plasma cells. Monoclonal components have been associated primarily with diseases of the plasma cell or lymphoproliferative system, such as multiple myeloma and Waldenstrom's macroglobulinemia. Monoclonal IgG<sub>1</sub> carbohydrate fragment (Fc) and the amino terminal (antigen-binding) end of the heavy chain subunit of the immunoglobulin molecule have been utilized in devices to complex the solid-phase antibody reagent. The sample, containing the analyte to be measured, is allowed to react with this immobilized antibody.

A suitable substrate for an enzyme-indicator system may then be used to determine the amount of the immunoglobulin analyte present in the sample.

Immunodeficiency disorders (hypoimmunoglobulinemias) necessitate immunoglobulin quantitation as an aid in diagnosis. Combined immune deficiencies, bearing on more than one immunoglobulin class and frequently on cell-mediated immunity as well, are characterized by the absence of serum IgG, IgM, and IgA. Selective immunoglobulin deficiencies are characterized by a decrease in one or two, but not all, of the immunoglobulin cases. Life-threatening infection is common in cases of immunoglobulin deficiency disorders. In the prototype of primary immune deficiency, Bruton's syndrome (10), there is a complete deficiency of all immunoglobulin classes and a striking susceptibility to pyrogenic infection with encapsulated bacteria. Late-onset immune deficiencies often present with infection, especially recurrent pneumonia and sinusitis, and immunoglobulin quantitation should be performed on any patients with chronic progressive bronchiectasis. In many situations, immunoglobulin production is unstable and concentrations may be variable from patient to patient and vary with time in the same patient. Diagnosis is also made difficult because clinical manifestations and susceptibility to infection do not always correlate. Therefore, repeated immunoglobulin quantitation at intervals is often necessary.

Patients with a selected deficiency of IgA have recurrent sinupulmonary infections, but these tend to be milder than in combined deficiencies and rarely result in abnormality of pulmonary function. Some patients with immunoglobulin deficiencies appear prone to developing autoimmune diseases. Rheumatoid arthritis and tenosynovitis are present in a 30-fold greater frequency than in the general population. Patients with IgA deficiencies in particular appear to show a tendency towards autoimmune disease, with rheumatoid arthritis, systemic lupus erythematosus, pernicious anemia, and thyroiditis (Grave's disease) being the most common.

Studies of atopic and nonatopic individuals have revealed no significant differences in serum concentrations of IgA, IgG, and IgM. Conflicting data have been presented with respect to serum IgD levels. Demonstration by Ishizaka (13) that skin-sensitizing antibody was associated with IgE prompted numerous studies of IgE in allergic disease. Various food, venom, inhalant, and parasitic allergens can induce IgE specific antibodies. It should be noted that in quantitating serum IgE, many factors other than the atopic state are important. The measurement of circulating IgE antibody, unattached to mast cells or basophils, may not provide the needed sensitivity necessary for a physician to begin initial immunotherapy (desensitization) procedures. The accepted format for determining atopy is by intradermal injection of an allergen (skin testing) into the patient's upper back. Measurement of the reaction (area of redness and swelling) provides a sensitive measurement of the patient's state of atopy. Elevated serum IgE levels have been found in patients with allergic asthma, combined allergy and allergic rhinitis. Although in most studies there is a wide range of overlapping values for IgE, the tendency in allergic disease is clearly toward higher levels.

Instrumentation for the agar diffusion, nephelometric, turbidimetric, electrophoretic, chemiluminescent, radiometric and bioluminescent measurement of immunoglobulins provide an interesting chronology of the sophistication of laboratory technology. In Table 1, a brief listing of these immunoglobulin measuring devices is provided to illustrate this progression in technology.

TABLE 1

**Examples of Submissions for Agar Diffusion, Nephelometric, Turbidimetric, Electrophoretic, Chemiluminescent, Radiometric and Bioluminescent Measurement of Immunoglobulins**

<b><u>Nephelometry</u></b>	<b><u>510(k) Number</u></b>
Abbott Laboratories-Bichromatic Analyzer	K771345
Behring Diagnostics, Inc.-Laser Nephelometer	K771603
Technicon Instruments Corp.-Fluoronephelometer III	K822341
Hyland Laboratories "PDQ" Laser Nephelometer	K770548
<b><u>Electrophoresis</u></b>	
Helena Laboratories	K860645
<b><u>Radioimmunoassay</u></b>	
Hybritech, Inc. (Eli Lilly and Cos.)	K802913
<b><u>Chemiluminescence</u></b>	
GEN-PROBE	K892216
London Diagnostics-Lumtag	K901075
<b><u>Bioluminescence</u></b>	
Vitek Systems	K881925
<b><u>Agar Gel Diffusion</u></b>	
Janssen Biochemical	K915650

### III. Specific Performance Characteristics

The FDA requests different types and amounts of data and statistical analyses to market in vitro diagnostic devices. The amount and types of data requested depend on the intended use and the technological characteristics of the new device. The data and statistical evaluation should be sufficient to determine if the device is substantially equivalent to a legally marketed device and is safe and effective for all claimed specimen type(s). Additional data may be necessary to substantiate certain intended use claims or establish an association of analyte levels with clinical condition of the patient. For all immunological test systems that measure immunoglobulins, each manufacturer must validate use of a new technology.

The reasons for requiring the determination of certain performance characteristics for in vitro immunoglobulin test systems are two-fold; to assess the influence of the prevalence of an imbalance in a particular immunoglobulin concentration (antigen specific vs total plasma levels) upon the clinical laboratory device's assessment of a patient's risk of having disease and the clinical laboratory procedures by which the poor detecting power of an in vitro device for relatively low prevalence condition may be improved. These assessments include clinical sensitivity (CSE) and clinical specificity (CSP). All test protocols for in vitro devices for physician's offices should be clearly stated with directions and the acceptance criteria for selecting clinical patients for inclusion in the study population; subjects should have indicated interest in participating in the study by having signed a patient consent form. 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. Results of the clinical study should be summarized and include explanations provided for unexpected results, any additional testing performed, and any other laboratory data or information which is relevant to the development of the final report. When appropriate, graphics of laboratory and clinical data should be provided in the form of receiver operating characteristic "ROC" curves, scattergrams, histograms, frequency distribution tables, for visual interpretation. Actual data (clinical and laboratory) may be requested.

Submission of the following data is required in order for FDA to make a determination of substantial equivalence:

Briefly discuss the salient concerns of the medical community including relevant medical/societal issues that may impact the review process or possibly the development of public policy. Discuss the principle of the test methodology. This comprises the sum of the error of measuring the test system against the blank reagent. Both measurements

are influenced by factors often encountered with new devices that employ microprocessor-controlled protocols. Software elements and microprocessor-controlled devices are often integrated systems for processing samples, calculating assay results and presenting interpretive categorization of the patient. See requirements for Moderate Level of concern in **Reviewer Guidance for Computer Controlled Medical Devices Undergoing 510 (k) Review** (available from the Division of Small Manufacturers Assistance, phone 1-800-638-2041). Analysis of method variance may be divided into two separate categories. For purposes of clarity, this guidance document will name one category "analytical performance characteristics", the latter will be termed "clinical performance characteristics". For new technologies, the application to the detection of immunoglobulins must be supported by literature references and other supporting reports of valid scientific investigations (6).

#### **A. Analytical Laboratory Studies**

For new technologies, a brief summary of prior methodologies used to detect the analyte, with specific parameters of importance to the operation of the new device should be provided, as well as analytical data determined with the device prior to testing in outside laboratories. Evaluative testing should be done within the manufacturer's facility or at a designated laboratory facility as part of the test development phase for the device.

##### **1. Validation of "Cut-Off"**

The device's expected values are derived from normative data that was accumulated from clinical studies using the device. For new technologies, data should demonstrate that the cut-off or action-level has been appropriately selected by testing the following:

- a. A minimum of 100 normal, healthy individuals (the manufacturer must provide a clinical description of this population) if the device is intended for screening purposes.
- b. A minimum of 100 individual patient specimens, most of which must have immunoglobulin levels above/or below the expected normal ranges, should be described in like manner. The 100 individual samples permit hypothesis testing: A specific level of an immunoglobulin in one population, associated with disease or clinical condition is determined and compared; other factors or clinical conditions are evaluated against this analyte level relative to age, gender or ethnic background of individual patients.

2. **Antisera characterization**

The specificity of the antisera, source of the antisera, either polyclonal or hybridoma derived, and sufficient information about the dilution of the antisera, filtration used and the standard curves generated from the antisera for each immunoglobulin assay, must be provided for FDA evaluation. The plasma components that might pose a cross-reactivity error should be investigated **using the assay system**. The test protocol should describe the procedures used to determine the amount of assay interference contributed by other plasma proteins, anticoagulants, other drugs or chemicals or differences in assay temperatures, time for assay incubation, washing of reactants, elapsed time of measurement of analyte and time of adding solutions that halt the assay procedure. The recommended frequency of preparing standard curves for the device should be stated. Parameters that should be discussed include the entire reagent set-up, diluting solution used, and the final dilution of serum samples examined for immunoglobulin concentration. A description of active and non-reactive ingredients (carriers) should be provided. Additionally, the reference preparation (calibrator) for each immunoglobulin should be identified.

3. **Limits of Detection.**

The analytical sensitivity (limits of detection) of the assay would be expressed relative to the detection level of analyte above two standard deviations of the test system's background or "blank"/threshold measurements. Specifications for the method of calculating results and procedures for curve fitting should be presented.

4. **Interference Studies.**

Any potentially cross-reacting or interfering substances potentially encountered in specific diseased patients should be tested using the assay system, e.g., drugs and/or their metabolites, oral contraceptives, that might commonly be used by patients tested for specific immunoglobulin type. Alternatively, a statement may be added to the Limitations section of the package insert that such testing has not been conducted for cross-reactivity or interference.

Verify that recommended storage conditions are compatible with the assay. State the optimal conditions based on specimen storage stability studies.

5. **Reproducibility and Repeatability Studies**

The National Committee for Clinical Laboratory Standards (NCCLS) recommended analysis of variance statistic that permits estimation of within-run and total standard deviations (SD) (14,15,16,17). Refer to EP-T2 (NCCLS Guideline) for recommended data collection formats and calculations. Perform separate calculations for each specimen tested for within-run and total precision.

For all test formats, a minimum of two negative, two low positive (containing less than normal level of immunoglobulin analyte) and two moderately high (elevated levels of immunoglobulin) serum specimens, in addition to controls included in the assay kit, should be tested three times in each of two runs on three different days. Serum specimens may be spiked specimens prepared by adding the purified reference immunoglobulin analyte to diluted transport media or specimen diluent.

Reproducibility studies should be performed on the same specimens at the two outside laboratories performing comparative studies, in addition to the manufacturer's laboratory. Controls provided in the test kit should also be included.

For single endpoint assays, provide percentage of results negative, borderline/equivocal, or positive for each set of tests.

For assays designed to be marketed in physician office laboratories, at least 30 different samples representing negative, low positive, and high positive specimens should be aliquoted, coded, blinded, and tested at 3 different physician office locations. Testing should be performed by office personnel who would perform the tests in these settings and compared to results obtained in a clinical laboratory setting. Additional data may be required.

For an analysis of method variance, determine the contribution to variance by the instrument used during the assay; results from comparing data reduction algorithms and user selected "curve-fitting" programs should be presented.

If dedicated instrumentation is used in specimen handling, or reading and interpreting results, use a different instrument at each site. If non-dedicated (individual) instruments are used, state specifications of instrument(s) used at each site.

**B. Clinical Comparison Studies**

It should be demonstrated by comparison that the performance of the device is substantially equivalent to another similar legally marketed assay in a well-controlled experiment. Two different and independent clinical sites with different populations (ethnic, gender, age diversity) should be tested. A description of test methods, including pertinent references and procedure protocols should be included in the submission. The comparison method used should be clearly described, including specimen collection methods, types of anticoagulants used, transit time between collection and assay, and storage conditions. Any other pertinent recommendations should also be described. Describe the component parts. The description of reagents should include instructions for handling radioactive materials, marketing format (whether in solution, lyophilized or absorbed onto or impregnated into a variety of solids), and function (primary reactant, calibrator, control, diluents, reaction enhancers, amplifiers, and "signal generators").

The new device under evaluation should be similarly described and should conform to procedures and recommendations specified in the product insert (16).

Provide package inserts for any commercially available assays tested in parallel or used to resolve discrepancies in testing results. Provide the names and telephone numbers of principal investigators and sites at which testing was performed. Comparative testing should be performed on an adequate number of positive and negative clinical specimens (following collection, storage, and testing instructions recommended in the package inserts), and the relative sensitivity and

specificity declared in the Performance Characteristics section of the package insert (17). The following are minimum recommended sample size populations for comparison testing of different specimen types:

1. Provide data and statistical analyses determined with the device to support performance parameters specific to and important for operating the device, e.g., reproducibility.

a. Justification of statistical methods

Avoid sole reliance on hypothesis testing (such as use of p values) which fails to give important quantitative information. Give the working data, statistical methods used with justified assumptions, test statistic results and the corresponding values. The use of specific statistical methods must be fully justified (e.g., parametric vs. non-parametric).

2. Antibody Sensitivity Studies. (Hybridoma-monoclonal antibody tests only) (3,9,10,12).

a. It should be demonstrated that an immunoglobulin-based assay be positive with blood samples from persons with commonly inherited immunoglobulin types. Any immunoglobulinopathy that is not detected should be listed in the limitations section of the package insert.

b. Humoral antibody to a mouse derived monoclonal antibody used during chemotherapy or visualization of tumor mass may prevent accurate measurement of an analyte and contribute to inaccuracies of analyte levels (12).

c. Immunologically related proteins.

It must be demonstrated that there is no test interference caused by the following immunologically active/related substances:

- i) blood group specific substances
- ii) immunoglobulin fragments (light chains: kappa and lambda), immunoglobulin fragments (Fab/c, Fd derived reagents)

3. Document symptomatic and asymptomatic patients and whether or not the disease process is related to infectious disease agents, plant, or animal allergens or to inherited traits of plasma immunoglobulins.

Additional parameters to be calculated are diagnostic efficiency within the context of clinical utility. Prove all claims for substantial equivalence and specific parameters for utilizing the device.

Explanations are required if clinically significant samples will be difficult to obtain, e.g., rare disease, few non-exposed persons because of widespread prevalence of patients with disease exposure early in life (chronic disease). For test systems that calculate risk-assessment factors from immunoglobulin levels or ratios of immunoglobulin isotype ratios, a discussion of the selection criteria as well as exclusion criteria applied to selected patient populations is required. Mathematical formulae, statistical procedures and their applicability and resolving power to the disease or condition, their ability to distinguish between affected and unaffected are necessary elements within the required explanations.

#### **IV. LABELING CONSIDERATIONS**

The following are additional details for some of the points in the statute [502(f)(1)] and regulations [21 CFR §809.10(b)].

##### **Package Insert**

##### **A. Intended Use.**

Describe the intended use based on the technology/methodology employed in the device. The following questions should be addressed:

1. What patient population should be tested?
2. What are the conditions and limitations of use of the device when used to diagnose specific immunoglobulin dyscrasia?
3. Whether the assay distinguishes subclass of immunoglobulins.
4. If the assay is used only with a special instrument.

A typical intended use statement is: \*\*\* test system is a device that consists of the reagents used to measure by immunochemical techniques the plasma (immunoglobulins A, G, M, D, and E) using ABC automated system for serum proteins.

##### **B. Detailed principle of the test methodology**

Include a complete description of the following components:

1. Description of the epitope(s) detected and/or quantitated.

2. Discussion should include the effect of non-specific binding of an enzyme conjugate to the stationary phase substance and the resulting high degree of variability. These combinations of characteristics create some difficulty in maximizing the signal-to-noise ratio with the objective of less false positive signal (15).
3. The end result or concentration of the analyte should be identified as micrograms per milliliter (ug/mL) or International Units per milliliter (IU/mL). Most often, IgE is measured and reported as IU/mL, whereas the other immunoglobulins are reported as ug/mL.

**C. Type of Specimen(s)**

The type(s) of specimens, serum or plasma, body fluid, cerebral spinal fluid (CSF) should be listed, along with any special considerations for collection of this specimen. Tables of "Expected Values" for each type of specimen should be presented.

**D. Conditions for Use**

Conditions for use of the device should describe any special applications of the device or specific contraindications or indications for use not addressed in the Intended Use statement, e.g., for cerebrospinal fluid samples only from infants as an aid in the diagnosis of infectious disease or trauma to spinal cord/brain. Unmodified immunoglobulin test systems are not recommended for synovial fluids, pleural fluid, or peritoneal washings.

**E. Expected Results**

1. Reference citations should be provided for assessing the prevalence of the immunoglobulin dyscrasia; incidence of abnormality in different populations and from appropriate specimen sites.

2. Indicate that prevalence may vary depending upon geographical location, age, gender of population studied, type of test system employed, specimen collection and storage employed, clinical and epidemiological history of individual patients and ethnic background of population tested.

F. **Limitations of the Test**

List important test limitations and all known contraindications, with literature references, when appropriate. Enzyme based double antibody test systems, which claim to detect individual immunoglobulin (heavy chain specific), must include the following in the Limitations Section, for analytes; (e.g., one antigen-binding domain of an immunoglobulin IgG molecule is described as the epitope to which the antibody binds). Additionally, recombinant deoxyribonucleic acid (DNA) technology allows innovative test systems to be designed; immunoglobulin fragments produced in specified bacterial cells (11), and purified Fab fragment reagents harvested that have the desired set of performance characteristics.

Four parameters (19) help us assess the probability of a correct result with an in vitro device; sensitivity, specificity, prevalence and diagnostic efficiency.

Predictive value of a positive "abnormal level" of a particular immunoglobulin or isotype of "immunoglobulin" and predictive value of a negative "level" or type of a particular "immunoglobulin" are secondary performance characteristics which are functions of CSE, CSP, prevalence, and should be calculated for the range of expected immunoglobulin based disease conditions or dyscrasias (based upon age, gender, lifestyle, geographic locality, ethnic origin, infectious agents, and other factors as well). The prevalence of disease or condition may be known, derived from calculations or publications in the medical literature. If prevalence of disease or condition is unknown, prevalence cannot be easily defined due to variations in geographic areas and definitions or criteria for disease.

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1. Interpretation of clinical in vitro devices, aside from clinical considerations, is based on the probability of the device's test result being within a given range of normal immunoglobulin concentrations.
2. Reliable results are dependent on adequate specimen collection. Specimen adequacy can only be assessed by phenotypic/genetic history of family members (in cases of immunoglobulinopathies).
3. For those assays using class-specific technology, a statement indicating that the assay will not specifically differentiate subclasses of the different immunoglobulins.

### I. Performance Characteristics

Summarize the data upon which the performance characteristics are based. Comparisons to legally marketed devices should also be presented, if performed.

1. Positive and negative predictive values should be based on specific populations sampled for each specimen type (serum, plasma, cerebrospinal fluid). State the prevalence determined at each testing site.
2. Present cross-reactivity studies in a tabular format, indicating negative, positive, and borderline/equivocal/indeterminate results for each Immunoglobulin class or Immunoglobulin fragment. Example of data presentation is given on the following page.

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$$\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN} + \text{Equivocals}}$$

$$\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP} + \text{Equivocals}}$$

Example of table for data presentation:

Test Results (Accepted Method)

		(Positive)	(Negative)	(Equivocal)	
T e s t	(Positive)	45 (TP)	95 (FP)	0	140
	(Negative)	5 (FN)	855 (TN)	0	860
	(Equivocal)	3	3	0	6
		53	953		1006

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3. Present units of measure and limits of detection for all immunoglobulin classes and subclasses, fragments of immunoglobulins or heavy or light chain designation.
4. Summarize reproducibility characteristics to include (e.g., precision, accuracy, linearity, analytical sensitivity) parameters that permit an acceptable level of test kit performance.
5. Present data from comparison studies, using separate categories for different immunoglobulin classes (18). All borderline/equivocal/indeterminate results should be clearly displayed. Discrepancies between test device and reference method may be resolved and presented as footnotes or presented in a separate table. Only those specimens which were further categorized (e.g., grouped by severity of allergy) should be represented.

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