**Review Criteria for In Vitro Diagnostic Devices for the Assessment of Thyroid Autoantibodies using Indirect Immunofluorescence Assay (IFA), Indirect Hemagglutination Assay (IHA), Radioimmunoasay (RIA), and Enzyme Linked Immunosorbent Assay (ELISA)**

This guidance was written prior to the February 27, 1997 implementation of FDA’s Good Guidance Practices, GGP’s. It does not create or confer rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both. This guidance will be updated in the next revision to include the standard elements of GGP’s.

REVIEW CRITERIA FOR IN VITRO DIAGNOSTIC DEVICES FOR THE ASSESSMENT OF THYROID

AUTOANTIBODIES USING INDIRECT IMMUNOFLUORESCENCE ASSAY (IFA), INDIRECT

HEMAGGLUTINATION ASSAY (IHA), RADIOIMMUNOASSAY (RIA), AND ENZYME LINKED

IMMUNOSORBENT ASSAY (ELISA).

This is a flexible document representing the current concerns and suggestions

regarding thyroid autoantibodies in vitro diagnostic devices employing IFA,

IHA, RIA, and ELISA methodologies. It is based on (1) current basic science,

(2) clinical experience, (3) the Safe Medical Devices Act of 1990 (SMDA) and

(4) FDA regulations in the Code of Federal Regulations (CFR). As advances are

made in science and medicine, these review criteria will be re-evaluated and

revised as necessary.

PURPOSE OF THE GUIDANCE DRAFT

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, quantitate and/or semi-quantitate thyroid autoantibodies in

clinical specimens can be cleared for marketing.

A premarket notification 510(k) submission provides 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 or

physician's office laboratories\* as an in vitro diagnostic test for the

qualitative, quantitative and/or semi-quantitative measurement of thyroid

autoantibodies by IFA, IHA, RIA or ELISA.

\*Devices may be cleared for use in physician's office laboratories when

additional data are submitted to demonstrate performance in these settings.

PRODUCT CODES: JNL, DDC, DDJ, JZO

REGULATION NUMBER:

21 CFR 866.5870 Thyroid autoantibody immunological test system.

Identification. A thyroid autoantibody test system is a device that consists

of the reagents used to measure, by immunochemical techniques, thyroid

autoantibodies (antibodies produced against the bodies own tissues).

Measurement of thyroid autoantibodies may aid in the diagnosis of certain

thyroid disorders, such as Hashimoto's disease (chronic lymphocytic

thyroiditis), nontoxic goiter (enlargement of the thyroid gland), and Graves'

disease (enlargement of the thyroid gland with protrusion of the eyeballs).

CLASSIFICATION: CLASS II (Performance Standards)

PANEL: Immunology (82)

REVIEW REQUIRED: Premarket notification (510(k))

I. CLINICAL INDICATIONS/SIGNIFICANCE/INTENDED USE

A. INTRODUCTION

The principle role of the thyroid gland is the storage and synthesis of

thyroid hormones. The normal thyroid gland is composed of numerous follicles,

each of which consists of a single layer of epithelial cells surrounding a

central lumen containing colloid. Thyroglobulin is the major component of the

thyroid follicular colloid. It is produced by the thyroid epithelial cells

and is a water soluble glycoprotein with a molecular weight of 670,000

daltons. Small amounts of thyroglobulin are present in serum. Microsomal

antigen is a 110,000 dalton glycoprotein present in the cytoplasm and on the

apical membrane of the thyroid cell. Reports have shown that thyroid

peroxidase (TPO) is the major component of the thyroid microsomal antigen and

is the antigenic component recognized by autoantibodies directed against the

microsomal glycoprotein. 1

Autoimmune thyroid disease is organ specific and is defined by the presence of

circulating antibodies. The most common antibodies seen are antibodies to

thyroglobulin and microsomal or thyroid peroxidase (TPO) of the thyroid

epithelial cell lining.

Autoantibodies to these thyroid antigens are detected in Hashimoto's disease

and its variants, Graves' disease, myxedema, nontoxic goiter, and thyroid

carcinoma. Antithyroglobulin and antimicrosomal (TPO) antibodies are seen

most often and are in the highest titers in Hashimoto's disease. Patients

with Graves' disease can also demonstrate thyroid antibodies of relatively

high titer. These autoantibodies are also seen in low titers in other thyroid

diseases and in individuals without clinical evidence of disease. 2

Two other thyroid antibodies less commonly seen are antibodies to the second

antigen of the colloid or CA-2 and human thyroid-stimulating immunoglobulin

(TSI). CA-2 is a minor protein of colloid distinct from thyroglobulin and

antibodies to it are of uncertain significance. Positive CA-2 reactions are

seen in a low percentage of thyroiditis patients in the absence of other

antithyroid antibodies and in 5 to 10 per cent of patients with Graves'

disease and thyroid cancer. 2 CA-2 antibodies can be detected by IFA. TSI,

previously referred to as long-acting thyroid stimulator (LATS), is a

polyclonal gamma globulin which appears to bind to a receptor on thyroid cells

and stimulates thyroid activity. It is present in the sera of about 50 per

cent of those with Graves' disease and is absent or present only in a small

number of patients with nodular toxic goiter or other thyroid disorders. TSI

can be measured by a competitive inhibition assay using 125I labeled TSH and

thyrotropin (TSH) receptors.

Hashimoto's thyroiditis is an inflammatory condition occurring in about 1 to 2

per cent of the population, mainly in middle-aged women, and is characterized

by gland enlargement as a result of marked lymphocytic inflammatory changes.

The latter may consist of lymphoid follicles with active germinal centers in

which much of the antithyroglobulin antibody appears to be synthesized.

Normal thyroid glandular structures are adversely altered, and in prominent

cases progressive disease may lead to thyroid atrophy and myxedema (dry, waxy

type of swelling, with abnormal deposits of mucin in the skin and other

tissues associated with hypothyroidism). In thyrotoxicosis, the thyroid may

contain small areas of lymphoid infiltration as well as evidence of the

typical glandular hyperactivity. Graves' disease is a multi-systemic

disorder, particularly in young to middle-aged females, consisting of varying

degrees of (1) hyperthyroidism with diffuse hyperplasia of the thyroid (the

most common pattern seen with diffuse toxic goiter); (2) a myopathy; and (3)

an infiltrative ophthalmopathy, frequently leading to exophthalmos (protrusion

of the eye ball). 2

As with all the organ-oriented diseases associated with autoantibody

production, it is important to determine when the antibodies under discussion

are pathogenic or are reacting to antigens liberated as a result of tissue

damage due to non-immune causes. Immune reactivity may not be the primary

pathogenic event but, once present, causes further tissue damage. Evidence

against a primary pathogenic role for thyroid autoantibodies in Hashimoto's

thyroiditis and Graves' disease is (1) the lack of correlation between the

level of autoantibody and the severity of disease in individual cases, and (2)

the lack of development of thyroid disease in infants with high levels of

antithyroid antibodies because of placental transfer.

B. INSTRUCTIONS:

Provide a concise discussion to include the following as appropriate. Support

the discussion with key literature citations.

1. Clinical indications, significance and intended use.

2. Background description of the thyroid disease involved including the

type of population affected (sex, age, etc.)

3. Significance of a positive result (disease indication and follow up

testing).

4. Significance of false positive and false negative results.

5. Salient concerns of the medical community including relevant medical

issues that may impact the review process or possibly the development

of public policy.

6. A brief historical summary of all test methodologies used to detect

the antibody(ies).

7. Merits/advantages and limitations/disadvantages of the device

methodology(ies) compared to other available methodologies.

8. Matrices.

II. DEVICE DESCRIPTION:

The determination of substantial equivalence is based on the specific intended

use (what analyte is detected and the indications for use) and the

technology/methodology utilized in the device. Discuss the principles of the

device methodology and whether it is well-established or new and unproven.

III. CLINICAL AND NONCLINICAL LABORATORY STUDIES: SPECIFIC PERFORMANCE

CHARACTERISTICS:

FDA requests different types and amounts of data and statistical analyses in

pre-market notification applications to market in vitro diagnostic devices.

The amount and type of data requested depends on the intended use,

technological characteristics of the new device, whether the test is

qualitative, quantitative or semi-quantitative and on certain claims made by

the manufacturer. The performance of the device can be established by

comparison to any legally marketed medical device (the predicate) with the

same intended use.

Prove all claims for substantial equivalence and specific performance

characteristics for using the device. Clearly document all protocols for in

vitro testing. Present test data results with analyses and conclusions.

Summarize results and include explanations for unexpected results and any

additional testing performed. Charts (scattergrams, histograms, etc.) may be

used as part of the analyses and conclusions when appropriate. Actual,

unprocessed laboratory data may be requested.

A. ANALYTICAL/LABORATORY/IN VITRO STUDIES

1. Validation of the Cut-off

Describe the rationale for determination of the assay cut-off(s).

Furnish descriptive information and laboratory data to show how the

cut-off point (distinction between positivity and negativity or

medical decision limit) was determined by the assay.

a. Define the population(s) used, including the following

information:

i. Number of samples in the normal population (used to

determine initial screening dilution) with samples

summarized according to gender and age groups. 4

ii. Number of specimens included in each disease group

summarized according to gender and age groups.

iii. Geographical area(s) from which the population was derived.

iv. Graphical (e.g., scattergrams, histograms, etc.)

representation of population characteristics.

b. Define the statistical method used to determine the cut-off

point(s).

c. Present a Receiver Operator Curve (ROC) analysis of cut-off point

selection and other graphical representations as appropriate.

d. Define the basis for the equivocal zone (if applicable).

2. Reagent characterization

a. Give a brief description of the antigen(s) and antibody(ies)

used in the assay.

b. If any recombinant technology was used in the preparation of the

antigen(s), describe method used.

3. Assay Specificity/Interfering Substances

Any potentially cross reacting or interfering substances encountered

in specific specimen types or conditions should be tested using the

assay system, e.g., hemolysis, lipemia, microbial contamination,

additional analytes or other autoantibodies present, and storage or

freeze-thawing.

a. Verify that recommended storage conditions are compatible with the

assay. State the optimal conditions based on specimen storage

stability studies. Both false positivity and negativity should be

evaluated (if applicable).

b. If the use of plasma is claimed, a study with each anticoagulant

must be performed to show that each anticoagulant does not

interfere with the assay.

i. For each anticoagulant, test 10 matched serum and plasma

specimens which are positive at the cut-off point.

ii. For each anticoagulant, test 10 matched negative serum and

plasma specimens.

Provide an explanation if interference from the anticoagulant is

not anticipated (e.g., high dilution factor).

4. Performance Characteristics

Include the following performance characteristics:

a. Analytical Sensitivity (if applicable)

The analytical sensitivity or detection limit is defined as the

lowest quantity differentiated from zero (95% confidence intervals

or 2 standard deviations (SD) above the mean of the Zero control

are commonly used). 4,5 Run the Zero standard (Zero diluent) at

least 20-25 times in the same run and calculate the mean of the

Zero standard and 2 SD of the mean (counts, OD's, etc.). If

levels of the analyte are not clinically significant,

determination of the detection limit may be irrelevant.

b. Relative Sensitivity and Specificity

The relative sensitivity and specificity as determined by

comparison to a legally marketed device or to a reference method

should be determined and reported in the Performance

Characteristics section of the package insert.

c. Linear Range

Validate the linear range of the assay with normal and abnormal

specimens covering the entire reportable range of the assay. 6

d. Reproducibility and Repeatability Studies 4,5,6,7,8

The National Committee for Clinical Laboratory Standards (NCCLS)

recommends an analysis of variance experiment testing two

clinically significant levels near medical decision limits (normal

or elevated) of an analyte, in this case thyroid autoantibodies. 8

Use controls simulating patient samples or actual patient

specimens 3 times in the same run and in two different runs each

day for 20 days. This permits separate estimation of between-day,

between-run and within-day standard deviations (SDs), as well as

within-run and total SDs.

i. Qualitative/Quantitative Tests:

Calculate total, between- and within-day and between- and

within-run means and coefficients of variation of

imprecision for each set of values.

ii. Semi-quantitative Tests:

In devices with a titration format, e.g., immunofluorescence

assays, demonstrate that intra-run reproducibility is within

the commonly accepted limits of plus or minus one two-fold

dilution.

iii. Means, SDs, and Coefficients of Variation:

Report in the Performance Characteristics section of the

package insert the appropriate means, SDs, and/or

coefficients of variation with confidence levels according

to number of times the sample is repeated. Report the

number of runs per day.

e. Prozone or High-Dose Hook Effect Studies

Test a sample with the highest titer available, serially diluted

and undiluted. If prozone problems are encountered, state in the

Performance Characteristics section of the package insert the

titer at which prozone problems were detected and a procedure for

the user to follow to correct the problem. Where appropriate,

describe the appearance of a prozone reaction for subjective

tests.

f. Alternative Testing Sites

Include reproducibility studies performed in these settings. In-site

testing for new technologies should include at least three

independent alternative testing sites. At each of the three

sites, the precision and accuracy of the device should be

evaluated. A statistically valid number of samples should be

tested by the site personnel and by professional laboratory

personnel, and the results compared, to show how the device

performs in the hands of the lesser trained user.

5. Comparison Studies

Compare the new device to a legally marketed device. Include the

package insert for the legally marketed device.

It is recommended that a recognized reference method (if available)

also be employed for comparison to enable a fair evaluation of the

proposed device's performance characteristics, particularly if there

are broad differences in methodology/ technology between the new

device and the legally marketed device.

a. Qualitative Tests:

The studies should be performed on an adequate number of positive

and negative specimens to support statistical significance. (An

appropriate number may be suggested by a statistician.)

b. Quantitative/Semi-quantitative Tests:

An assay is considered quantitative only if a recognized reference

material of known concentration is available for standardization

of a calibrator or standard used in the assay to calculate

results.

If the same reference material is used in the new device as in the

predicate device to substantiate the quantitative claim,

comparison data should be presented to show correlation between

the two assays when running the reference material as samples.

Run the serially diluted reference material by the new device and

the predicate device. The assays should show similar results.

Compare results obtained using positive thyroid autoantibody

samples free from interfering substances from 40-100 persons

covering the whole assay range (from low to high levels of

antibodies). 7,9

Perform a linear regression analysis and report the slope,

intercept, correlation coefficient, the assay range, and the

nature and number of samples tested.

c. Comparison Discrepancies:

Equivocal results or discrepancies between the new device and the

comparison method should be resolved using another method or

clinical diagnosis.

6. Specimen Collection and Handling Conditions

State specimen collection, storage and handling conditions in the

package insert and provide data or appropriate literature references

in the submission to substantiate claims.

7. Computer Controlled Medical Devices

For information regarding computer assisted clinical laboratory

devices, refer to "Review Guidance for Computer Controlled Medical

Devices Undergoing 510(k) Review" available from the Division of

Small Manufacturers Assistance (DSMA), 1-800-638-2041.

B. CLINICAL INVESTIGATIONS

In certain instances it is necessary to require comparative clinical data

to establish substantial equivalence, e.g., a new or unfamiliar methodology

or technological feature is introduced in a device category in which

clinical performance is claimed to be equivalent to a legally marketed

device using "conventional" technology.

For 510(k) submissions, perform a comparison of the device to a legally

marketed device. Ideally this study should be done at independent clinical

laboratory site(s). A minimum of two additional independent investigators

at separate outside locations is recommended. The investigators should be

identified by institutional name and address.

1. Adequate Clinical Investigations

a. Prove all claims for substantial equivalence and specific

parameters for using the device.

b. Describe all protocols for clinical studies and consistently

adhere to the protocols.

c. Determine the sample size, prior to beginning the study, that will

be statistically sufficient to determine whether or not the device

is safe and effective.

d. Sampling Method:

Describe sampling method used in the selection and exclusion of

patients.

i. Patient selection:

Include samples from individuals with diseases or conditions

that may cause false positive or false negative results with

the device. Ideally, a prospective study is preferred.

However, if a retrospective study is used, include all

eligible patients who meet the patient selection criteria as

specified in the protocol.

ii. Account for all patients and samples. Insure that data

points are included for every sample for every patient.

2. Establishing Reference Ranges

a. Normal individuals:

Establish a normal reference range with a statistically sufficient

number of samples from normal persons characterized by age, sex,

geographical location and any other factors that would influence

the values obtained. 3,10

b. Patient groups:

i. Confirm that the new device detects the percentage of

positives generally expected for each disease for which the

device is intended. Use a statistically sufficient number

of patients characterized by age, sex, geographical

location, any symptoms of disease, clinical presentation,

and any other factors that would influence the values

obtained. 3,10

ii. False results:

Patients positive for antinuclear antibodies (ANA) could

give a false positive result in an IFA assay for the

detection of thyroid autoantibodies.

Radioisotopes administered to the patient for diagnostic or

therapeutic purposes may interfere in some RIA assays.

Provide reports, if any, of false positive and false

negative results for each disease as appropriate.

c. Sample Types Claimed:

Investigate all sample type(s) claimed in the intended use

statement unless other data proves that there is no difference

between them.

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

Include the package insert for the new thyroid autoantibody device. Support

the statements throughout the document with key literature citations.

A. INTENDED USE

Briefly describe the intended use based on the technology/methodology used

in the device. Include the following information:

1. Whether the assay is qualitative, quantitative, or semi-quantitative.

In order to claim to provide a quantitative result, the calibrators

or standards must be calibrated to a reference material of known,

established value.

2. Test methodology.

3. Specimen type(s).

4. Indicate if the device is for use in clinical laboratories and/or

alternative care sites. The Limitations section should include any

specific training required for test performance.

A typical Intended Use statement would be:

"ABC's \*\*\* test system is a device for the semi-quantitative measurement of

anti-thyroglobulin antibodies by indirect immunofluorescence in human serum

to aid in the diagnosis of certain thyroid disorders, such as Hashimoto's

disease, nontoxic goiter, and Graves' disease."

B. QUALITY CONTROL (QC)

The package insert should recommend levels of quality control samples and

their number, matrix type, placement and interpretation to ensure that the

system meets its performance claims. Include a statement that if controls

do not behave as expected, assay results are considered invalid and should

be repeated.

Controls should be handled in the same manner as patient samples. For

example, if the patient sample is diluted or titered the control material

should also be diluted or titered using the same diluent.

C. RESULTS

Give an adequate description of expected results and interpretation.

1. IFA

a. Give a description of the fluorescence for a positive and negative

result.

b. List possible staining patterns which may be found. Photographs

or diagrams may be helpful.

c. Give pattern description and interpretation as it relates to the

particular antibodies.

d. Give instructions for titering positive samples to end point.

2. IHA

a. Describe in detail the appearance of a positive and negative

result.

b. Give a definition and description of the end point for a positive

result.

c. Describe the appearance of a prozone reaction (antibody excess)

and instruct the user what to do if prozoning is suspected.

d. Explain the procedure for repeating samples which have results

above the linearity of the assay.

3. RIA

a. Explain the procedure for manual (if applicable) calculation of

percent bound for each sample and include a sample calculation.

b. Give instructions for plotting percent bound versus concentration

of the standard showing examples of typical results (numerical and

graphical).

c. Give a brief explanation of how automated calculations are

performed, e.g., the type of data reduction program used.

4. ELISA

a. Explain the procedure for calculating the value of the unknown

including a sample calculation.

b. Explain the procedure for repeating samples which are above the

linearity of the assay. Give instructions for dilution of samples

including the dilution factor and type of diluent to be used.

D. LIMITATIONS OF THE PROCEDURE

Include a statement of limitations of the procedure to include the

following:

1. A statement that the test result in and of itself is not diagnostic

for thyroid disease and should be considered in conjunction with

iodine uptake and other standard thyroid tests and the clinical

presentation of the patient.

2. IFA

a. Explain possible variations between different types of

fluorescent microscopes.

b. Give warnings concerning distinguishing the thyroid specific

cytoplasmic fluorescence from that obtained with

mitochondrial antibody in primary biliary cirrhosis. If

mitochondrial antibody is suspected, the distinction can be

made by running more specific tests for antinuclear

antibodies.

c. Multiple antibodies may be present and complicate the

staining interpretation. Serially diluting the patient

sample will often aid in distinguishing multiple patterns.

d. A prozone reaction can appear as a doubtful positive or

negative because of a small amount of antigen in relation to

the large amount of antibody present. If prozone is

suspected, the patient sample should be serially diluted.

3. IHA

Give warnings concerning heterophile antibodies and a possible

prozone reaction

4. RIA

a. Give warnings concerning possible interference from

radioisotopes administered to the patient for diagnostic or

therapeutic purposes.

b. Supply instructions for proper disposal of radioactive

materials.

E. EXPECTED VALUES

1. The expected value in the normal population is negative. However,

apparently healthy, asymptomatic individuals (5-10%) may test

positive for thyroid autoantibodies. The incidence of these

antibodies increases with increasing age beginning in the seventh

decade for women and the eighth decade for men.

2. Thyroid autoantibodies may be present in non-thyroid disorders such

as pernicious anemia, diabetes mellitus, Addison's disease, and

Sjogren's syndrome.

3. Present information showing the incidence or prevalence of each type

of thyroid autoantibody for each disease state.

From: Division of Clinical Laboratory Devices

Immunology Branch

Prepared by: Deborah M. Moore, Scientific Reviewer

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