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REVIEW CRITERIA FOR ASSESSMENT OF ANTI-NUCLEAR ANTIBODIES (ANA) IN VITRO DIAGNOSTIC DEVICES USING INDIRECT IMMUNOFLUORESCENCE ASSAY (IFA), IMMUNODIFFUSION (IMD), AND ENZYME LINKED IMMUNOSORBANT ASSAY (ELISA).

This is a flexible document representing the current concerns and suggestions regarding ANA in vitro diagnostic devices employing IFA, IMD, 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 and/or semi-quantitate anti-nuclear antibodies (ANA) 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 alternative testing sites* as an in vitro diagnostic test for the qualitative and/or semi-quantitative measurement of ANA by IFA, IMD, or ELISA.

* Devices may be cleared for use in alternative testing sites when additional data are submitted to demonstrate equivalent performance in these settings.

PRODUCT CODES: LRM, LSW, KTL, LKO, LKP, LJM, LKJ, DHN, LLL, DHC

REGULATION NUMBERS:

A. 21 CFR 866.5100

Identification. "An anti-nuclear antibody immunological test system is a device that consists of the reagents used to measure by immunochemical techniques the autoimmune antibodies in serum, other body fluids, and tissues that react with cellular nuclear

constituents (molecules present in the nucleus of a cell, such as ribonucleic acid (RNA), deoxyribonucleic acid (DNA), or nuclear proteins). The measurements aid in the diagnosis of systemic lupus erythematosus (a multisystem autoimmune disease in which antibodies attack the victim's own tissues), hepatitis (a liver disease), rheumatoid arthritis, Sjogren's syndrome (arthritis with inflammation of the eye, eyelid, and salivary glands), and systemic sclerosis (chronic hardening and shrinking of many body tissues)."

B. 21 CFR 866.5820

Identification. "A systemic lupus erythematosus (SLE) immunological test system is a device that consists of the reagents used to measure by immunochemical techniques the autoimmune antibodies in serum and other body fluids that react with cellular nuclear double-stranded deoxyribonucleic acid (DNA) or other nuclear constituents that are specifically diagnostic of SLE. Measurement of nuclear double-stranded DNA antibodies aids in the diagnosis of SLE (a multisystem autoimmune disease in which tissues are attacked by the person's own antibodies)."

CLASSIFICATION: Class II (Performance Standards)

PANEL: Immunology (82)

REVIEW REQUIRED: Premarket notification (510(k))

I. CLINICAL INDICATIONS/SIGNIFICANCE/INTENDED USE

A. INTRODUCTION

Antibodies to nuclear antigens (ANA) are found in the serum of patients with a variety of autoimmune diseases. ANA have also been called "auto-antibodies" since they are antibodies produced by an individual against their own tissue antigens. Over the last several years, investigators have found that there are many types of ANA that react with different cellular nuclear components. Generally, the nuclear antigens may be divided into three categories: the nucleic acids (DNA and RNA), the histone (basic) proteins and the nonhistone (acidic) proteins. More recently, the differentiation of ANA into antibodies with different immunologic specificities have been found to be important in the diagnosis and management of patients with systemic rheumatic diseases. The occurrence of anti-nuclear antibodies with diverse specificities suggests that different systemic rheumatic diseases are induced by different etiologic mechanisms. ^{1,2,3}

Recent studies of autoantibodies have supplied information on the molecular structure of their homologous autoantigens. This new information may help determine their function. This is important because many of these nuclear autoantigens are involved in essential or important functions of cells, such as DNA replication, transcription, and protein synthesis, and anti-nuclear autoantibodies have been shown to inhibit such functions. ^{3,4,5}

Antibodies to double stranded DNA (dsDNA), also called native DNA (nDNA), and denatured or single stranded DNA (ssDNA), histone, nuclear ribonucleoprotein (RNP) and Smith antigen (Sm) all occur in SLE, while antibodies to Sjogren's Syndrome A (SSA)/R^o* and Sjogren's Syndrome B (SSB)/L^a* occur in SLE and Sjogren's syndrome (SS). Antibodies to Jo¹-1-Topoisomerase occur in polymyositis, while antibodies to scleroderma-associated antigen (SCL-70) and centromere occur in patients with progressive systemic sclerosis (PSS). ⁶ Some of the antigens such as SSA/Ro, SSB/La, Sm, RNP, Jo-1, and SCL-70 were originally termed extractable nuclear antigens (ENA).

The indirect immunofluorescent assay (IFA) technique was described for the study of ANA in 1957. ⁷ Since then the IFA has proved to be invaluable in the detection of ANA. A negative result on the IFA generally rules out the possibility of SLE if the patient is not undergoing steroid or immunosuppressive therapy, but as many as 5 % of SLE patients may be ANA negative by IFA. ⁸

IFA will not detect all possible ANA since negative results have been observed in patients with Sjogren's syndrome (SSA/Ro), polymyositis (Jo-1), and scleroderma, although such patients demonstrate specific precipitin antibodies by other immunological tests. ⁶ Autoantibodies to SSA/Ro and Jo-1 may exhibit a cytoplasmic pattern by IFA and can also be shown by IMD. ^{2,3}

The primary antigen-antibody binding tests, such as IFA and ELISA are the most sensitive. The tests involving secondary reactions such as IMD are less sensitive but are valuable in the specific identification of nuclear antibodies such as anti-Sm, anti-RNP, anti-Jo-1 and anti-SCL-70. ¹

In general, the ANA lack tissue or species specificity and they cross react with nuclei from different sources. Recent developments have demonstrated that it is important not only to detect the presence of the ANA but also to identify the quantity and immunologic specificities of the ANA in a given patient. The identification of the immunologic specificity (Sm, RNP, etc.) of the ANA helps in the differential diagnosis of rheumatic disease and in the management and treatment of patients. ^{1,3}

* Represent the first two letters of the patient's name whose serum was used to identify the reaction in agar diffusion.

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 rheumatic/connective tissue diseases 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/societal 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:

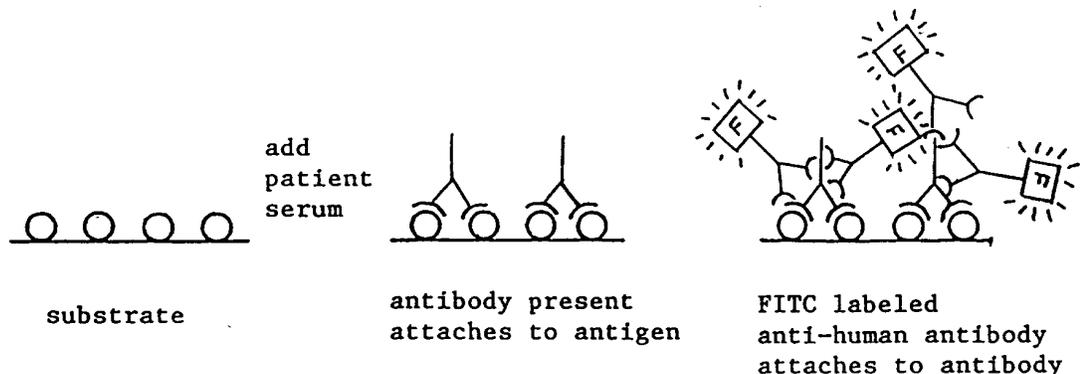
The determination of substantial equivalence is based on the specific intended use (what analyte is detected and the indications for use) and the technology/methodology utilized in the device. Discuss the principles of the device methodology and whether it is well-established or new and unproven. The following descriptive information should be included in a 510(k) submission.

A. INDIRECT IMMUNOFLUORESCENCE ASSAY (IFA)

The IFA technique was adapted to ANA testing and is generally considered the method of choice for ANA screening and semi-quantitation.⁷ It is now recognized that many sources of nuclear material may be employed as a substrate for ANA testing. Although most of the original ANA research was performed using rat or mouse liver or kidney cryopreserved tissue sections, the

use of human or animal tissue cell culture substrates have provided a reliable and easy to interpret alternative substrate for ANA testing. These cultures provide cells in different stages of development (mitosis). Mitotic figures aid in differential pattern recognition as well as in detecting previously unreported anti-nuclear antibodies. It is important to know the substrate employed, as serum reported as negative for ANA when cryopreserved tissue is used may be positive using a cell line, thus explaining some of the interlaboratory variations seen. Other influencing factors are the type of substrate fixatives and buffer solutions used as some nuclear antigens may be destroyed by certain fixatives. SSA/Ro may be lost when alcohol or methanol is employed, while others such as SSB/La and RNP, which are extremely soluble in buffer solutions, may be washed out during the assay and give false negative results. ² The hemoflagellate Crithidia luciliae has been found to be particularly useful in identifying antibodies to dsDNA.

Patient samples and controls are incubated with antigen substrate to allow specific binding of ANA to cell components. If ANA are present, a stable antigen-antibody complex is formed. After washing to remove unbound serum proteins, the substrate is incubated with an anti-human antibody conjugated to fluorescein isothiocyanate (FITC). When results are positive, a stable three-part complex consisting of fluorescened antibody bound to human anti-nuclear antibody bound to nuclear antigen is formed.



This complex can be visualized with the aid of a fluorescent microscope. In positive samples, the cell nuclei will show a bright apple-green fluorescence with a staining pattern characteristic of the particular nuclear antigen distribution within the cells. If the sample is negative for ANA, the nucleus will show no clearly discernable pattern of nuclear fluorescence.

A serum is considered positive for ANA if the nucleus shows specific fluorescence greater than the negative control, and a clearly discernable pattern of staining can be seen in a majority of the cells. Some sera may demonstrate nuclear and cytoplasmic fluorescence with no apparent nuclear staining pattern. The phenomenon is generally due to heterophile antibodies and should be reported as negative for ANA. ⁹

Four main types of staining patterns are seen with positive ANA tests. ¹⁰ These various patterns and the basis for them are as follows:

1. Homogeneous: A solid staining of the nucleus, with or without apparent masking of the nucleoli, due to antibodies reactive with dsDNA, deoxyribonucleoproteins (DNP), and/or histone. High titers are suggestive of SLE; lower titers are suggestive of SLE as well as other connective tissue diseases.
2. Peripheral (rim): A solid staining, primarily around the outer region of the nucleus, with weaker staining toward the center of the nucleus due primarily to antibodies directed against dsDNA. High titers are suggestive of SLE; lower titers are suggestive of SLE as well as other connective tissue diseases.
3. Speckled: A fine to coarse grainy appearing staining dispersed throughout the nucleus due to antibodies directed against nuclear antigens RNP, Sm, SS-B or SCL-70. High titers are suggestive of SLE (Sm antigen), mixed connective tissue disease (RNP antigen), scleroderma (Scl-70 antigen), or Sjogrens syndrome-sicca complex (SS-B antigen); lower titers may be suggestive of other connective tissue diseases.

The centromere pattern seen on HEp-2 and other cell culture substrates is a discrete speckled staining pattern highly suggestive of the CREST syndrome variant of scleroderma or progressive systemic sclerosis. ^{3,9}

4. Nucleolar: Large coarse speckled staining of the nucleolar membrane due to antibodies reactive with RNA-nucleoprotein complexes. High titers are prevalent in scleroderma and Sjogren's syndrome.

Sera from patients with SLE often contain multiple autoantibodies which react with different nuclear antigens. Thus, patterns of nuclear staining may often be mixed patterns rather than distinctly of one type or another. Also, these multiple autoantibodies may be present in different concentrations in the patient's serum and on serial dilutions different patterns of nuclear staining may be observed. ¹⁰

Fluorescent intensity may be scored by following the guidelines for fluorescent antibody reagents. ¹¹

- 4+ Brilliant yellow-green (maximal fluorescence): clear-cut nuclear outline; sharply defined nuclear structure or component.
- 3+ Less brilliant yellow-green fluorescence: clear-cut nuclear outline; sharply defined nuclear structure or component.
- 2+ Definite nuclear pattern with dim fluorescence: nuclear structure or component less well defined.
- 1+ Subdued fluorescence: nuclear outline almost indistinguishable from nuclear structure or component in the majority of the cells.

All ANA positive sera should be semi-quantitatively assayed by end point dilution determination. This is accomplished by making 2-fold serial dilutions of all positive sera and controls. The end point titer is the highest dilution that produces a positive reaction.

B. IMMUNODIFFUSION (IMD)

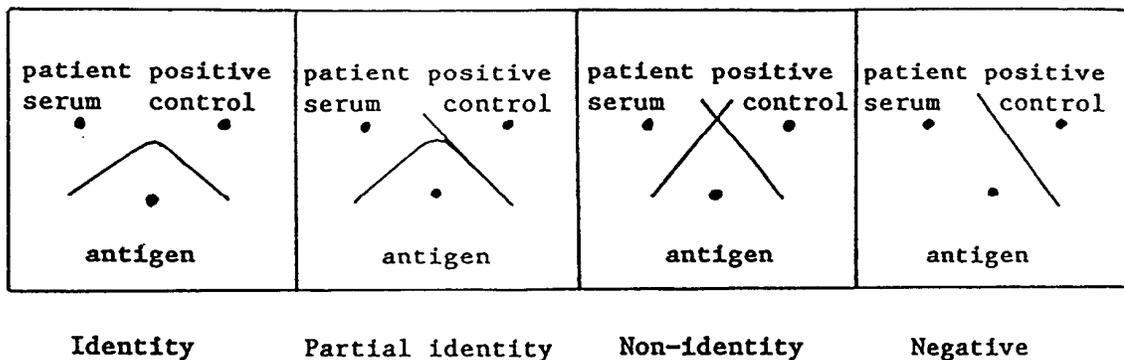
IMD requires 18 to 48 hours of incubation and reading of immunoprecipitin lines of identity, partial identity, and nonidentity. It is a subjective technique which requires trained and experienced technical staff.

The central well of the agarose plate is filled with a multispecificity nuclear antigen. Patient samples and controls are placed in the wells surrounding the antigen well. After incubation for 18 to 24 hours at room temperature, a line of precipitation forms in the agarose gel where nuclear antigen diffuses and meets homologous antibody that has also diffused. Samples that appear negative after 24 hours should be incubated an additional 24 hours and read again.

Proper interpretation of patient results depends on clear resolution of the precipitin lines that form between patient sera and nuclear antigen wells. Determination of the patient antibody specificity depends on proper interpretation of precipitin lines that form between patient sera and adjacent control wells.

1. Identity: Precipitin lines that form a continuous line or arc between patient and control indicate each serum is reacting with identical nuclear antigens.

2. Partial identity: Precipitin lines that form a "spur" off the arc between patient and control wells indicate patient and control are reacting with an identical antigen but the patient sera contains an additional antibody that reacts with an additional antigen in the agar that does not react with the control sera.
3. Non-identity: Precipitin lines that cross between patient and control indicate each serum is reacting with a different nuclear antigen.
4. Negative sera do not produce precipitin lines.

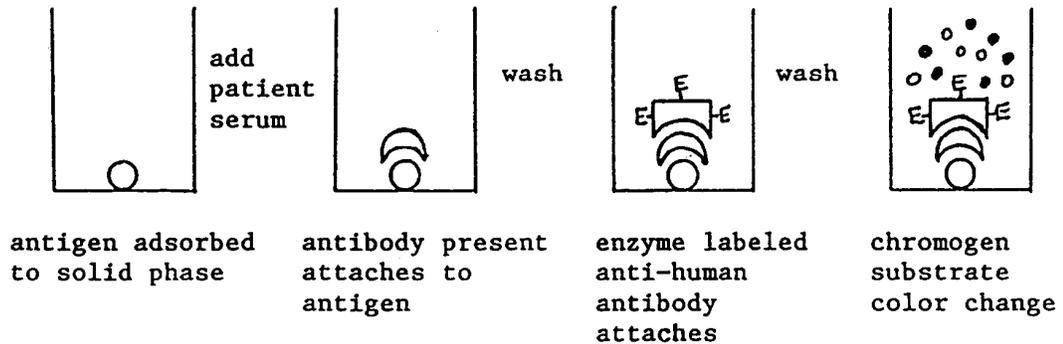


C. ELISA

During the past several years ELISA performed with antigens isolated from natural tissue sources increasingly have been used to detect ANA.¹² ELISA utilizes a purified antigen that will adhere to plastic microtiter plates. The sensitivity and specificity of these newer methods have been demonstrated by correlation with previously used methods and by the increased rate of autoantibody detection in patients with rheumatic diseases. Moreover, recent studies with antigens produced by recombinant DNA technology in microtiter plate ELISA procedures have also shown good sensitivity and specificity.¹³ ELISA methodology removes the subjectivity of IFA interpretation.

The general procedure for ELISA is the same for most of the auto-antibodies. Purified antigen is bound to the solid phase microtiter plate. Diluted patient's sera are added to the wells and incubated at room temperature. If specific ANA are present they bind to the antigen and do not rinse off during the washing step. Unbound antibodies and other serum proteins are washed off. Subsequently, when enzyme-labeled anti-human antibody is

added to the reaction well and incubated, it binds to any bound ANA. The unbound conjugate is removed by washing and the enzyme (chromogenic) substrate is added. The enzymatic reaction is stopped after incubation to fix the intensity of the color development.



The specimens containing ANA produce a color endpoint reaction which is read photometrically using an ELISA plate reader. The intensity of the reaction is directly proportional to the amount of bound enzyme conjugated antibody which in turn is directly proportional to the amount of ANA in the patient sample.

Results are calculated by comparison to a calibration standard that is assayed with each batch of tests. Results are reported in either ELISA units per milliliter (EU/mL) or in International Units per milliliter (IU/mL) when international standards have been established.

ELISA is an objective technique to detect the presence or absence of autoantibodies and also provides a semi-quantitative measurement that may be useful in determining the level of ANA present.

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 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).

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 raw 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 comprising the normal population (used to determine initial screening dilution) with samples summarized according to gender and age groups. ¹⁴
 - 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.
- 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. Prevalence in the Asymptomatic Population

Establish the prevalence of ANA in the asymptomatic population(s) using the specified cut-off point.

- a. Assay a statistically significant number of specimens which are representative of the intended use, clinical utility, and matrix of the new device. Furnish results from the new device and results from a pre-existing device or a published study from the same general population performed with a device of the same methodology.
- b. Summarize the distribution of the population according to age group, geographical area, and the number of positive, negative, and equivocal results.

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, 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.
- 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.

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 above the mean of the zero control are commonly used).^{15,16} Run the Zero standard (Zero diluent) at least 20-25 times in the same run and calculate the mean of the Zero Standard and two standard deviations (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.¹⁷

d. Reproducibility and Repeatability Studies

^{15,16,17,19,20}

The National Committee for Clinical Laboratory Standards (NCCLS) recommends an analysis of variance experiment testing two clinically significant levels near medical decision limits (subnormal, normal, or elevated) of an analyte, in this case ANA.²⁰ Use controls simulating patient samples or actual patient specimens 2 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 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, 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.

f. Alternative testing sites

Include reproducibility studies performed in these settings. On-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 less 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. Semi-Quantitative tests:

Compare results obtained using positive ANA samples free from interfering substances from 40-100 persons covering the whole assay range (from low to high levels of ANA). ^{19,21}

Perform a linear regression analysis and report the slope, intercept, correlation coefficient, the assay range, and 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.

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. Plan Adequate Clinical Investigations

- a. Prove all claims for substantial equivalence and specific parameters for using the device.
- b. Describe all protocols for clinical studies.
- c. Plan 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.

2. Establishing Reference Rangesa. Normal individuals:

Establish a normal reference range with a statistically sufficient number of samples from normal persons characterized by age, sex, geographic location and any other factors that would influence the values obtained. ^{14,22}

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. ^{14,22}

ii. False results:

Certain drugs, including procainamide and hydralazine, may induce a lupus erythematosus-like disease. ³ Patients with drug induced LE may demonstrate positive homogeneous or homogeneous/peripheral ANA's commonly directed against nuclear histones. SLE patients undergoing steroid treatment may have negative test results. Positive ANAs are also seen in a small percentage of patients with infectious and/or neoplastic disease. ²³

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 ANA 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 or semi-quantitative.
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 antinuclear antibodies by indirect immuno-fluorescence in human serum to aid in the diagnosis of systemic lupus erythematosus (SLE)."

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.

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 particular disease states.

2. IMD

- a. Give a narrative and pictorial description of typical precipitin line formation.
- b. Give an explanation of identity, non-identity, partial identity, and negative.
- c. Explain the special situation encountered with the anti-Sm and anti-RNP complex.
- d. Give instructions for what steps to follow if precipitin lines to other nuclear antigens are encountered e.g., proliferating cell nuclear antigen (PCNA), rheumatoid arthritis nuclear antigen (RANA), etc.

3. 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 auto-immune disease and should be considered in conjunction with other laboratory test results and the clinical presentation of the patient.
2. Possible reasons for false positives e.g., certain drugs including procainamide or hydralazine, or false negatives e.g., prozoning or the masking of nuclear patterns by cytoplasmic antibodies.
3. For IFA procedures explain possible variations between different types of fluorescent microscopes.

E. EXPECTED VALUES

1. The expected value in the normal population is negative. However, apparently healthy asymptomatic individuals (1-3%) age 20-40 years may contain ANA in their sera. This percentage increases with aging, particularly in the seventh decade of life (2-5%). Give possible exceptions and situations where normal persons could have positive results.
2. Present information showing the incidence or prevalence of each type of ANA for each disease state. See attached chart "Characteristics of Anti-Nuclear Antibodies."

V. BIBLIOGRAPHY

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**CHARACTERISTICS OF
ANTI-NUCLEAR ANTIBODIES**

AUTO-ANTIBODY TO:	ASSOCIATED DISEASE	INCIDENCE	IFA PATTERN
DNA			
	ds DNA	SLE	Few Cases
both	ds DNA	SLE	75-90%
	ss DNA	high levels	homo., rim, both
	ss DNA	SLE	85-90%
		Drug induced LE	70-80%
		Rheum. arthritis	50-60%
		hepatitis	50-60%
			Not detected by routine screening
DNP	SLE, LE cell	55-60%	homo., rim
	Drug induced LE	60-70%	
HISTONES	SLE	25-30%	homo., rim, both
	drug induced LE	>95%	
Sm	SLE	25-30%	speckled
RNP	MCTD	95-98%	
	SLE	40-45%	speckled
	scleroderma	20-25%	
	Rheum. arthritis	10%	
SSA/Ro	Sjogrens-sicca	50-60%	Not detected by routine screening (cytoplasmic)
	SLE	25-30%	
	"ANA neg" SLE	40-50%	
SSB/La	Sjogrens-sicca	40-50%	speckled (cytoplasmic)
	SLE	10-15%	
	"ANA neg" SLE	40-50%	
NUCLEOLAR	Scleroderma	40-50%	
	SLE	25-28%	Nucleolar
	Rheum. arthritis	10%	
CENTROMERE	CREST var Sclero.	80-90%	Discreet, speckled (cell culture subs)
	Raynaud's	15-20%	
SCL-70	Scleroderma	20-25%	Atypical speckled
JO-1	Polymyositis	25-30%	cytoplasmic

MCTD - Mixed Connective Tissue Disease; SLE - Systemic Lupus Erythematosus