

Guidance for Industry and FDA Staff

Recommendations for Anti-Nuclear Antibody (ANA) Test System Premarket (510(k)) Submissions

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This document supersedes “Review Criteria for Assessment of Anti-Nuclear Antibodies (ANA) In Vitro Diagnostic Devices Using Indirect Immunofluorescence Assay (IFA), Immunodiffusion (IMD), and Enzyme-Linked Immunosorbent Assay (ELISA)” issued September, 1992

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Preface

Public Comment

Written comments and suggestions may be submitted at any time for Agency consideration to the Division of Dockets Management, Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD, 20852. Alternatively, electronic comments may be submitted to <http://www.regulations.gov>. Please identify your comments with the of this guidance document. Comments may not be acted upon by the Agency until the document is next revised or updated.

Additional Copies

Additional copies are available from the Internet at: <http://www.fda.gov/cdrh/oivd/guidance/848.pdf>. You may also send an e-mail request to dsmica@fda.hhs.gov to receive an electronic copy of the guidance or send a fax request to 240-276-3151 to receive a hard copy. Please use the document number 848 to identify the guidance you are requesting.

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This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for, or on, any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance.

1. Introduction

This guidance is intended to provide information for device manufacturers and FDA staff concerning premarket submissions for various types of *in vitro* diagnostic tests for anti-nuclear antibodies (ANA). The document is a revision of the guidance “**Review Criteria for Assessment of Anti-Nuclear Antibodies (ANA) in Vitro Diagnostic Devices Using Indirect Immunofluorescence Assay (IFA), Immunodiffusion (IMD), and Enzyme-Linked Immunosorbent Assay (ELISA)**,” issued in 1992. It is updated to address issues associated with new developments for ANA testing. This guidance document is meant to address *in vitro* diagnostic devices that detect antinuclear antibodies as an aid in the diagnosis of autoimmune disease. It is not meant to address tests that differentially diagnose autoimmune diseases (see Section 4–Scope in this guidance document for the classification regulation and product codes for ANA test systems).

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

The Least Burdensome Approach

The issues identified in this guidance document represent those that we believe should be addressed before your device can be marketed. In developing the guidance, we carefully

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considered the relevant statutory criteria for Agency decision-making. We also considered the burden that may be incurred in your attempt to follow the guidance and address the issues we have identified. We believe that we have considered the least burdensome approach to resolving the issues presented in the guidance document. If, however, you believe that there is a less burdensome way to address the issues, you should follow the procedures outlined in the “A Suggested Approach to Resolving Least Burdensome Issues” document. It is available on our Center web page at: <http://www.fda.gov/cdrh/modact/leastburdensome.html>.

2. Background – Premarket Submissions

A manufacturer who intends to market a device of this generic type must conform to the general controls of the Federal Food, Drug, and Cosmetic Act (the act), including the premarket notification requirements and obtaining a substantial equivalence determination from FDA prior to marketing the device (see 21 CFR 807.81 and 807.87).

This document supplements other FDA documents regarding the specific content requirements of a premarket notification submission. You should also refer to 21 CFR 807.87, the guidance - Format for Traditional and Abbreviated 510(k)s¹ and the section of CDRH’s Device Advice webpage entitled Premarket Notification 510(k).² As explained in “**The New 510(k) Paradigm – Alternate Approaches to Demonstrating Substantial Equivalence in Premarket Notifications; Final Guidance**”³ a manufacturer may submit a Traditional 510(k) or has the option of submitting either an Abbreviated 510(k) or a Special 510(k). A manufacturer may choose to submit an abbreviated 510(k) when a guidance document exists, when special controls have been established, or when FDA has recognized a relevant consensus standard. Manufacturers considering certain modifications to their own cleared devices may lessen their regulatory burden by submitting a Special 510(k). For more information on types of Premarket Notification 510(k)s that may be submitted to FDA, see CDRH’s Device Advice webpage, **Premarket Notification 510(k)**.

3. Scope

The scope of this document is limited to devices identified and classified under 21 CFR 866.5100 Antinuclear antibodies immunological test system:

(a) Identification. An antinuclear 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, deoxyribonucleic acid, or nuclear proteins). The measurements aid in the diagnosis of systemic lupus erythematosus (a multisystemic autoimmune disease in which antibodies attack the victims own tissues), hepatitis (a liver disease), rheumatoid arthritis, Sjögren’s syndrome (arthritis with inflammation of the eye, eyelid, and salivary glands), and systemic sclerosis (chronic hardening and shrinking of many body tissues).

¹ <http://www.fda.gov/cdrh/ode/guidance/1567.pdf>

² <http://www.fda.gov/cdrh/devadvice/314.html>

³ <http://www.fda.gov/cdrh/ode/parad510.pdf>

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(b) Classification. Class II (performance standards).

The following is a list of current product codes for this device type:

- LRM – Anti-DNA Antibody (Enzyme-Labeled), Antigen, Control
- LSW – Anti-DNA Antibody, Antigen and Control
- KTL – Anti-DNA Indirect Immunofluorescent Solid Phase
- MQA – Anti-Ribosomal P Antibodies
- LKO – Anti-RNP Antibody, Antigen and Control
- LKP – Anti-Sm Antibody, Antigen and Control
- OBE – Anti-ss-a 52 autoantibodies
- LJM – Antinuclear Antibody (Enzyme-Labeled), Antigen, Control
- LJK – Antinuclear Antibody, Antigen, Control
- DHN – Antinuclear Antibody, Indirect Immunofluorescent, Antigen, Control
- NYO – Autoantibodies, anti-ribonucleic acid polymerase (rnap) iii antibody
- LLL – Extractable Antinuclear Antibody, Antigen and Control

4. Device Description

We recommend that you identify your device by regulation and (where appropriate) the product code described in Section 3, above. You must identify a legally marketed predicate device. 21 CFR 807.87(f). In addition, we recommend that you include the following:

- A description of the device and its intended use. The description should include the device design, components, and methodology.
- Relevant literature references that support your claims for the device.
- Identification of the risk analysis method(s) used to assess the risks associated with your device and a discussion of how you have addressed the risks.
- If your device includes instrumentation, you should describe the instrument and software and demonstrate the performance of the instrument with your test.

5. Performance Characteristics

General Recommendations

We recommend you address the following when demonstrating the performance of your device:

- Include patient samples or sample pools derived from the intended use population (i.e., patients diagnosed with autoimmune disease and age-matched normal controls) for the various analytical protocols described below. Although spiked samples can be

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used to supplement the analytical studies, FDA cautions against using only spiked samples in the evaluations because spiked samples may not provide an accurate assessment of your test's performance.

- Perform all of your studies in accordance with the procedures you plan to recommend to users in the labeling.
- Provide specifics about the protocols used in the studies. You should include information about the particular Clinical Laboratory Standards Institute (CLSI) protocols or guidelines that you followed, and a description of any specific aspects of the protocols that you modified.

Specific Performance Characteristics

Precision/Reproducibility

Quantitative/semiquantitative ANA tests:

A test that reports a numerical result may be quantitative or semi-quantitative. You may claim that your antinuclear antibody (ANA) test is quantitative if the test is standardized to a recognized reference material. We consider ANA tests that are not standardized to a reference material (and thus have results reported in arbitrary units) to be semiquantitative.

For semi-quantitative and quantitative ANA tests, we recommend that you characterize within-run, between-run, and total precision using patient samples or sample pools. Protocols provided in "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline" CLSI document EP5-A2 are generally suitable for use. The document includes guidelines for experimental design, computations, and a format for stating performance claims.

We recommend that you evaluate precision, including reproducibility at relevant ANA concentrations over the reportable range of the assay and near the medically-relevant decision point (cut-off) that distinguishes a positive from a negative result. You should describe the following associated with your precision studies:

- Sample matrices tested.
- Protocol for evaluating precision, e.g., number of days, runs and replicates in each run cycle.
- Target concentrations.
- Computational methods for calculating precision results. Results should include standard deviation (SD) and % CV observed for within-run, between-run, and total precision, as well as your pre-defined acceptance criteria for precision.

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- The procedure and materials used for calibration, and an evaluation of control materials, as appropriate.
- For assays that employ new technology, or use subjective interpretation for determining the result, you should evaluate lab-to-lab reproducibility across three sites. You should provide a description of the sites, the number of operators at each site, and the protocol performed at each site.

Qualitative ANA tests:

Qualitative ANA tests produce binary report results, e.g., a positive or negative result, rather than a numerical result. We recommend you follow CLSI document EP12-A “User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline,” to establish an estimate of precision for these types of tests. Generally, you should demonstrate reproducibility using both positive and negative samples that are very near the cutoff. You should describe all relevant aspects of your study design, including the parameters listed in the bullets above, as appropriate.

Analytical Specificity–Interference

We recommend that you characterize the effects of potential interferents on assay performance. Examples of experimental designs, including guidelines for selecting interferents for testing, are described in CLSI Document EP7-A “Interference Testing in Clinical Chemistry; Approved Guideline.”

Potential sources of interference can include anticoagulants, preservatives, antibodies (e.g., human anti-mouse antibodies (HAMA)), and compounds normally found in serum, such as triglycerides, hemoglobin, and bilirubin. You also should determine whether your particular assay is prone to interference from other sources such as common medications that are likely to be used by the particular patient population for which the test is intended.

We recommend that you provide the following information associated with your interference studies:

- Sample matrices. There are unique concerns for interference depending on the sample specimen. Your interference study should address the types of interference relevant to the sample matrix for which your assay is intended.
- Specific interferents tested and their concentrations in the sample. Typically, interference studies involve adding the potential interferent to the sample and determining any bias in the recovery relative to a control sample without interferent added. Frequently, however, the metabolic byproducts of medications are sources of interference and therefore samples obtained from people who are taking the medication under evaluation for interference are preferred.

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- Concentrations of ANA in the samples. You should evaluate interference in samples with ANA concentrations close to the cut-off.
- Method of computing interference, the acceptance criteria for % bias, and the results. We recommend that you identify any observed trends in bias (i.e., negative or positive) and indicate the range of observed recoveries in the presence of the particular interferent. This approach is more informative than listing average recoveries alone.

We recommend that you state the conditions under which interference does not occur and include this information in your labeling. For example, “No interference from rheumatoid factor (RF) at [specify concentration] was seen in samples containing [specify concentration] ANA.”

Analytical Specificity–Cross-reactivity

We recommend you evaluate assay specificity by testing sera from patients with known autoimmune syndromes and infectious diseases, and demonstrate that your assay does not cross-react with other autoantibodies and infectious disease antibodies. You should describe the study design and results of the evaluation in your 510(k).

Prozone Effect (Hook Effect)

You should demonstrate the effect of high antibody titers on your assay and state in the package insert the highest antibody concentration that can be evaluated without a hook effect. We recommend you describe any controls you have implemented to alert the user that the sample may need to be diluted and re-assayed.

Linearity

For quantitative or semi-quantitative tests, we recommend that you characterize the linear range of the assay by evaluating samples with known concentrations of autoantibody. In your description of the study design, we recommend that you include the sample types and preparation, concentrations, the number of replicates tested and statistical methods used. You should state your acceptance criteria and present the data to demonstrate you have met it. We recommend that your data include the slope and intercept of the linear regression line with 95% confidence intervals, the range of linearity, and the deviations from linearity that were observed across the range. We also recommend that you include observed values relative to the expected values for each level evaluated.

Analytical Sensitivity (Limit of Detection)

If your ANA test is quantitative, you should report the limit of blank (LoB) and the limit of detection (LoD) for your assay. We recommend that you follow the definitions and study design recommended in CLSI Document EP17-A “Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline,” to obtain these estimates.

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Expected values/Cut-off

You should describe the rationale for determination of the clinical decision point (assay cut-off) for your assay. This should include the statistical method used to determine the number of samples you evaluated in both the normal population and the diseased population. If possible, we recommend you provide a summary of the age, gender and demographics of the patients evaluated. You should demonstrate that your cut-off appropriately distinguishes positive and negative samples. If your assay has an equivocal range, you should define the basis for the equivocal result and indicate how samples with equivocal results should be handled, for example, whether samples with an equivocal result should be retested. You should define the statistical method used to determine the cut-off and present Receiver Operator Curve (ROC) analysis, as appropriate.

Calibrators and Control Material

We recommend that you provide the following information about the calibrators and controls in the assay kit:

- Protocol and acceptance criteria for real-time and accelerated stability studies for opened and unopened calibrators and controls. This should include the methods or analyses you used and your criteria for acceptable recovery at the expiration date.
- Protocol and acceptance criteria for value assignment and validation of the various calibrator and control levels. This should include the methods and analyses used.
- Traceability to a domestic or international standard reference material. FDA recommends that anti-DNA assays be standardized to the WHO reference standard Wo80.

For information about calibrators marketed separately as class II devices under 862.1150, see the guidance “Abbreviated 510(k) Submissions for *In Vitro* Diagnostic Calibrators,” <http://www.fda.gov/cdrh/ode/calibrator.html>.

Specimen collection and handling conditions

You should substantiate that the recommendations in the labeling for specimen storage and transport produces consistent results over the recommended storage times and temperatures. For example, an appropriate study may include an analysis of aliquots stored under the conditions of time, temperature, or allowed number of freeze/thaw cycles recommended in your labeling. You should state your criteria for an acceptable range of recoveries under the recommended storage and handling conditions.

6. Method Comparison

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General Recommendations

You should compare results obtained with your device to those obtained with a predicate device with similar indications for use and include a comparison to a recognized reference standard (if available). Comparison to a reference standard will 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 predicate device.

We recommend that you evaluate patient samples with ANA concentrations distributed across the reportable range of the assay, including samples near the cut-off concentration. We recommend that you provide a statistical justification to support the study sample size that you used. Banked (retrospective) samples may be appropriate for some studies provided information characterizing the samples is available (e.g., gender, age, clinical diagnosis). You should provide a clear description of the inclusion and exclusion criteria used for sample selection.

When providing the results of the method comparison study, we recommend that you include the following information:

For Quantitative/Semi-Quantitative Tests: Plots of results from the new assay (y-axis) versus the predicate (x-axis), including all of the data points, the estimated regression line and the line of identity. Data points should represent individual measurements. We recommend that you employ Deming regression, Passing-Bablok, or another method that accounts for variability in both test systems. You should provide a description of the analytical method used to fit the regression line and results of regression analysis including the slope and intercept with their 95% confidence limits, the standard error of the estimate (calculated in the y direction), and a correlation coefficient.

For Qualitative Tests: A 2 x 2 table showing agreement between the new assay (rows) versus the reference method or predicate device (columns), and calculations of the percent positive, percent negative and overall agreement between the methods, including the 95% confidence intervals. If your test or the predicate employs an equivocal zone, you should state how you designated those results in the table and include that information in the description of results.

You should provide the line data in your submission and follow guidelines provided in CLSI, Document EP9-A2 "Method Comparison and Bias Estimation Using Patient Samples; Approved guideline." You may contact OIVD for input on your proposed study plan prior to initiating your studies.

Special Considerations for Anti-DNA Antibody Assays

Anti-double stranded DNA (dsDNA) antibodies

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The measurement of anti-dsDNA antibodies is an important aid in the diagnosis of systemic lupus erythematosus (SLE). Positive results from the antinuclear antibody test or double stranded DNA (dsDNA) test are included by the American College of Rheumatology (ACR) as two of the possible 11 criteria for SLE classification. (See the ACR web site for more information at <http://www.rheumatology.org/>).

There are several factors that cause variation in the level of anti-dsDNA antibodies in a patient sample. Anti-dsDNA antibodies are heterogeneous with respect to avidity, immunoglobulin class, cationic charge, and complement fixing ability. There can also be variation caused by the clinical status of the patient depending on the type of autoimmune disease and the disease activity at the time the sample is obtained (e.g., increased levels in disease progression and transition, followed by decreased levels in exacerbation or flare due to antigen/antibody complexes taking the level of anti-dsDNA antibodies out of circulation).

Of significant concern, with respect to FDA review of your assay's performance, are differences in performance that might be observed in a method comparison study between your assay and the predicate, due to variations in methodologies between the two assays. Such variations in methodologies can include:

- Source of the dsDNA antigen, e.g., biological sources such as calf thymus or *Crithidia luciliae*, or synthetic antigens;
- Impurity of the antigen, e.g., contamination from residual proteins following purification, or the presence of single stranded DNA (ssDNA);
- Presentation of the capture antigen, such as:
 - purified and in solution, e.g., Farr assay detects high avidity dsDNA antibodies, and detects both IgG and IgM dsDNA antibodies;
 - attached to a solid phase in an ELISA or EIA method that detect both anti-dsDNA high and low avidity antibodies; or
 - native state as with the whole organism *Crithidia luciliae* mounted on a glass slide in IFA.

To help address these concerns and help overcome these differences, we recommend evaluating a reference material (e.g., WHO Wo80) with a known concentration of anti-dsDNA antibodies, in a side-by-side comparison of your device with the predicate device. The results should be analyzed and presented using Deming regression statistics or other similarly appropriate statistical regression analysis method. You should include the slope and y- intercept with 95% confidence intervals for each, and the resulting graph and scatter plots along with the line data in the 510(k).

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If your assay labeling includes claims to measure high avidity anti-dsDNA antibodies, you should describe how you demonstrated the assay measures only high avidity antibodies. We also suggest that you compare results of your assay to the Farr assay.

Anti-single stranded DNA (ssDNA) antibodies

If the new device includes claims to measure anti-ssDNA antibodies, you should describe the antigen, including the source and method of production, e.g., biological source produced by heat denaturing calf thymus dsDNA, or synthetic source by synthesizing ssDNA (oligonucleotides of known sequence). If the capture antigen is also capable of binding anti-dsDNA antibodies, a statement that the result interpretation for anti-ssDNA antibodies assay must be made in conjunction with the anti-dsDNA result (detects antibodies to both ssDNA and dsDNA), should be part of the Indications for Use and Intended Use statements. You should include a discussion of the difficulties related to measuring and interpreting anti-ssDNA antibodies results in the labeling.

Clinical Sensitivity and Specificity

If your assay detects an ANA analyte for which there is minimal or no regulatory history with the FDA (i.e., a “new” analyte), you should demonstrate in your premarket submission how you established clinical performance, including clinical decision points. In these cases, we recommend that you submit data from clinical studies conducted to support the use of the analyte in the target population(s), and in which a percentage of patients are expected to be positive for the antibody (clinical sensitivity). We also recommend testing the performance of the analyte in sera from patients with other autoimmune disease antibodies, with infectious disease antibodies and in age and sex matched normal subjects where the analyte results are generally expected to be negative for the antibody (clinical specificity). These study groups should be used to establish and validate the cut-off and equivocal zone (if applicable) for the assay. We highly recommend that you contact OIVD for feedback concerning your planned studies and/or submit a proposed study protocol for review prior to beginning your studies to support the 510(k).

7. Labeling

The premarket notification must include labeling in sufficient detail to satisfy the requirements of 21 CFR 807.87(e). Final labeling must also comply with the requirements of 21 CFR 809.10 before a medical device is introduced into interstate commerce. Some of these requirements are further discussed below. The following suggestions are aimed at assisting you in preparing labeling that satisfies these requirements.

Intended Use

You must specify the product’s intended use. 21 CFR 809.10(a)(2), (b)(2). The intended use should be representative of the target populations tested, and the performance characteristics of the assay.

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Principle of the Method

You must specify the principles of the procedure. 21 CFR 809.10(b)(4). You should include a clear and concise description of the technological features of the specific device and how the device is to be used with patient samples.

Directions for Use

You must provide a step by step outline of recommended procedures. 21 CFR 809.10(b)(8). Include clear and concise instructions for the assay procedure. You should include a validated procedure for dilution if you instruct users to dilute samples with values above the highest calibrator. You should instruct the user to repeat samples with results in the equivocal or gray zone using the same sample or a fresh sample collected at a later date. Instructions should encourage local/institutional training programs designed to familiarize users with the features of the device and how to use it in a safe and effective manner.

Quality Control

The step by step outline of the procedure must include details of kinds of quality control procedures and materials required, as well as details of calibration. 21 CFR 809.10(b)(8)(v) and 21 CFR 809.10(b)(8)(vi). You should ensure that the specifics of calibration and quality control procedures you recommend to users are those necessary to ensure performance claims.

Limitations

You must include a statement of limitations of the procedure. 21 CFR 809.10(b)(10). We recommend that you thoroughly discuss the limitations of the assay, and include the following, as appropriate:

- A statement that the performance of the assay was not established in the pediatric population.
- A statement that a negative result does not rule out the possibility of an autoimmune disease.
- A statement that some patients may have autoimmune antibodies and show no clinical signs of disease.
- A description of any possible sources of interference, such as from heterophilic antibodies, e.g., human anti-mouse antibodies (HAMA).
- A statement that the test result, in and of itself, is not diagnostic for autoimmune disease and should be considered in conjunction with other laboratory test results and the clinical presentation of the patient.
- Possible reasons for false positive results, e.g., certain drugs including procainamide or hydralazine; or false negative results, e.g., prozoning or the masking of nuclear immunofluorescent staining patterns when multiple antibodies are present.

Performance Characteristics

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You must include specific performance characteristics of the assay. 21 CFR 809.10(b)(12). We recommend that you provide the results and brief description of the protocol for each performance characteristic discussed in Sections 5 and 6. You should include information cited in those sections that would be relevant to aid the user in understanding test performance.

For quantitative assays, you should summarize data of your regression analysis for the method comparison study; including number of samples, slope, intercept, standard error, correlation coefficient and the concentration range tested. For qualitative assays, you should show the 2x2 table with percent positive, percent negative and overall agreement.