

# Harmonization of Autoimmune Diagnostics with Antinuclear Antibody Testing Algorithm: Approach of Appropriateness and Clinical Relevance

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**KEY WORDS:** diagnostic algorithm, autoimmune rheumatic disease, antinuclear antibodies (ANA), anti-extractable nuclear antigens (ENA), anti-double stranded DNA (dsDNA)

*IMAJ* 2014; 16: 640–642

Limiting inappropriate test requests and identifying a balance between available economic resources and growing health needs is crucial for health care services today. In this context, harmonizing testing algorithms is a goal. As such, the clinical laboratory plays a critical role to help interpret clinical presentations that are often misleading in these pathologies. Recent advances in diagnostic technologies for autoimmune diseases have had a vital impact on the diagnostic approach to these pathologies.

The presence of antinuclear antibodies (ANA) in the serum, anti-extractable nuclear antigens (ENA) and anti-double stranded DNA (dsDNA) is one of the diagnostic criteria for autoimmune rheumatic disease. The test request for these assays has grown exponentially, due to increasing knowledge of the pathogenetic and diagnostic value of autoantibodies in autoimmune diseases and the inappropriate use of laboratory diagnostics. The latter problem is not precisely defined as yet, and is mainly a consequence of several factors, including inadequate collaboration or audit between physicians and laboratory personnel, availability of different techniques and methodologies in laboratory practice for assessing the same marker, and lack of a uniform terminology and diagnostic algorithms when performing autoantibody testing. Furthermore, a reduction in the number of clinically inappropriate requests and the establishment of a reasonable balance between available economic resources and increasing needs is a principal target of health care services worldwide.

To improve the appropriateness of the test requests in autoantibody testing, reliable and universally accepted diagnostic algorithms need to be defined and implemented; these algo-

gorithms should have been developed using the available guidelines found in the current scientific literature and should be shared by all physicians working in clinical immunology. The most appropriate strategy for requesting autoimmune rheumatic disease laboratory testing should encompass selective criteria; it should begin from a clinical suspicion, followed by a logical succession of analyses performed with sensitive tests at an early stage and specific tests for confirmation.

In view of its solid diagnostic performance, the ANA test has been proposed by several authors as a first-level test in the laboratory diagnosis of autoimmune rheumatic diseases, whereas tests for antibodies to specific nuclear antigens can only be detected when ANA screening is positive or if the patient has clear signs and symptoms suggesting a systemic rheumatic disease (second-level tests).

## METHODS

In the light of this background, we conducted a multicenter study in the northwestern region of Emilia Romagna, supported by a regional grant for innovative research projects during the years 2008–2010. This observational research aimed at comparing the number of ANA, anti-dsDNA and anti-ENA testings as well as the percentage of positive test results before and after implementation of the diagnostic algorithm in hospitalized patients, performing ANA at the first level. A multidisciplinary team consisting of clinical immunology and laboratory scientists was established to collect and analyze diagnostic criteria, clinical needs, laboratory report formats, analytical procedures, as well as the number of tests performed. The laboratory results and the clinical protocol were both validated by clinical data emerging from the clinical follow-up studies. With regard to cost/management efficiency in terms of the number of tests performed, a significant reduction in the number of anti-dsDNA (26%) and anti-ENA (15%) tests was observed when comparing the production statistics of the first term of 2008 with those of 2009, whereas the ANA determination increased by 10%, following the trend of increasing requests recorded for autoimmunity tests.

Regarding the diagnostic specificity of the algorithm, the percentages of positivity observed for the second-level tests increased after application of the diagnostic algorithm, particularly for the ENA test (13% vs. 17%). This is attributed to the fact that this test can be more easily standardized in the various centers as compared with the anti-dsDNA tests (9% vs. 11%). As expected, the percentages of positivity did not significantly change for the ANA test since it was used as the “baseline” test.

## RESULTS

Following this multicenter study, on 1 March 2013 the Emilia-Romagna region accepted and adopted through legislation the diagnostic ANA algorithm (creating a new test called ANA Reflex) with the aim of promoting the appropriate use of laboratory investigations. This will serve as a common guide for autoantibody testing, placing the ANA test at the first level with subsequent steps to be carried out directly by the laboratory. This algorithm has since been implemented in every autoimmune laboratory in the region, for both hospitalized patients and outpatients. Figure 1 depicts the diagnostic protocol.

### THE PROTOCOL SPECIFICALLY IMPLIES THAT:

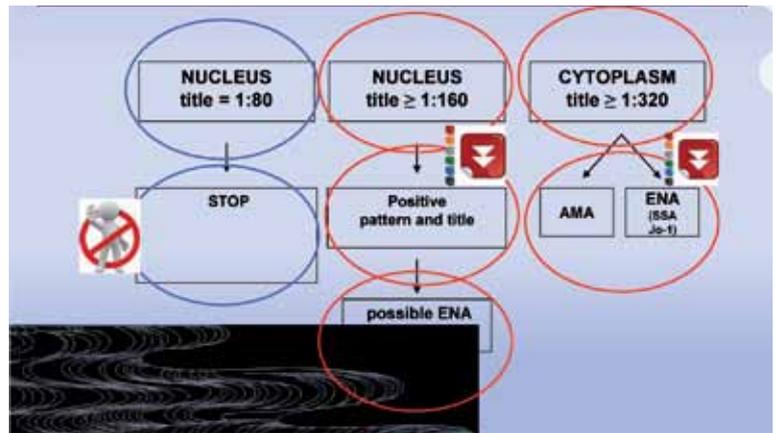
- The ANA test is the first-level test performed by an indirect immunofluorescence assay on HEp-2 cells. The screening dilution used is 1:80. In our clinical protocol we also suggest that the ANA test be performed only when there is a consistent clinical suspicion of autoimmune rheumatic disease
- When ANA testing is negative or positive to dilution < 1:160, no additional tests for antibodies to specific nuclear antigens (anti-dsDNA and anti-ENA) should be performed. These tests, even when not requested, are performed when ANA is positive for dilution ≥ 1:160 with any fluorescence pattern. These tests are performed even if the ANA test is negative, when a specific clinical request has been sent to the laboratory (request form or computerized note on request file).

During the first year of ANA Reflex use we observed that the requests for ANA Reflex represented a significant percentage of total ANA requests in both Parma and Modena (46% and 49% respectively). We also found that ENA requests during this period showed a reduction of 22% in Parma and 21% in Modena, accompanied by a substantial increase in positivity. DNA showed a reduction of 14% in Parma and 26% in Modena before and after implementation of ANA Reflex, with a substantial improvement in selected positive cases.

The definition and application of a diagnostic algorithm may help in test requesting and interpretation of laboratory findings throughout the challenging patient management.

In our experience, a diagnostic protocol including both screening and confirmation tests should allow a cascade of

Figure 1. ANA algorithm: Emilia Romagna region



tests in the diagnosis of autoimmune rheumatic disease, thereby improving the appropriate use of tests for specific autoantibodies. Moreover, the inappropriate use of immunological tests can lead to misdiagnosis, inappropriate therapy, and waste of health care resources.

### CONCLUSIONS

Close collaboration and audit between clinicians and laboratory personnel will enable standardization and widespread implementation of diagnostic algorithms for a more efficient use of immunological tests in the diagnostic evaluation, prognostic assessment, and monitoring of patients with systemic rheumatic diseases.

The percentage of second-level test positivity increased for both ENA and dsDNA after implementation of the diagnostic protocol. Furthermore, the introduction of this diagnostic algorithm led to a significant decrease in the number of second-level tests.

Efficiency and efficacy are strongly linked. We expect that the clinical protocol for autoantibody testing as presented in this article will be effective in improving both patient outcome (i.e., efficacy) and efficiency over a broad geographic area.

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