Antinuclear Antibodies: Measurement by Multiplex Immunoassay

Antinuclear antibodies (ANA) refer to the antibodies that target nuclear constituents of cells, such as the nuclear membrane, nucleoplasm, nucleoli, and nuclear organelles. Measurement of ANA is extensively used for diagnosing and monitoring various autoimmune diseases such as systemic lupus erythematosus (SLE), Sjögren’s syndrome (SS), scleroderma (Scl), mixed connective tissue disease (MCTD), polymyositis (PM), and dermatomyositis (DM). The traditional methods for detecting ANA are indirect immunofluorescence (IIF) on epithelial (HEp-2) tissue and enzyme immunoassay (EIA). The IIF method allows the detection of a large number of autoantibodies that bind to a variety of nuclear antigens from all the cellular constituents listed above. A positive ANA screen leads to further laboratory investigations on the presence of markers useful for diagnosis of autoimmune diseases such as anti-dsDNA antibodies or anti-extractable nuclear antigens (anti-ENA) antibodies. These techniques used in the identification of autoantibodies are time-consuming, laborious and characterized by frequent discrepancies in results between laboratories. Moreover, IIF testing requires highly trained personnel and its standardization is difficult particularly due to the subjective interpretation of specimens. Therefore, there is a need to select a suitable strategy for simultaneous multiple autoantibody analysis in order to support the clinical diagnosis more effectively. Multiplex immunoassay test methods have recently been introduced in the clinical laboratory. Recent reports suggest that automated Luminex-based systems can rapidly and efficiently determine a profile of multiple antibodies having clinical significance. These systems utilize a new approach for the simultaneous measurement of autoantibodies based on multiple-dyed (fluorescent) beads coated with specific antigens and flow cytometry detection. Considerable data confirm the advantage of this new technology and its application in diverse fields of medicine. An application of multiplexed technology in the field of autoimmunity suggests that this assay is a useful tool for the detection of ANA and extractable nuclear antigens in autoimmune diseases.

The BioPlex 2200 ANA screen (by BioRad) is a fully automated Luminex-based system developed for high-throughput analysis of 13 autoimmune analytes simultaneously in a single tube. This system allows the simultaneous detection in one sample of 13 autoantibodies {reacting with SSA (52 and 60 kDa), SSB, Sm, Sm/RNP, RNP-A, RNP-68 kDa, Scl70, centromere B, dsDNA, chromatin, Jo1, ribosomal P proteins}. This system has been successfully evaluated for detection of autoantibodies in the sera of healthy subjects by measuring the positivity rate of 510 samples collected from a cohort of apparently healthy blood bank donors. The positivity rate per individual analyte ranges from 0.0% to 1.8%, which translates into individual analyte specificity ranging from 98.2% to 100%. Another retrospective study with a cohort of 1,104 patient samples demonstrates that this system is suitable as a sensitive screening test to confirm or to exclude the presence of a large number of autoantibodies simultaneously and rapidly. A multicenter, prospective clinical study demonstrated agreement between multiplex and EIA testing ranged from 99% (95% CI 98% to 100%) for Jo-1 to 70% (95% CI 76% to 82%) for ANA. The medical decision support software (MDSS) algorithm suggested an appropriate disease association in 75% to 100% of patients with SLE. The MDSS is included in the Bioplex system to suggest a disease association based on how individuals with similar autoantibody values had been diagnosed. The MDSS uses a database of 1,200+ real-world individuals and contains the specific BioPlex ANA screen results (all 13 auto-antibodies)
plus the autoimmune disease diagnosis as determined by a physician (if disease was present) for each individual included. These findings suggest that patterns of autoantibodies detected by this system, when analyzed by an interpretative algorithm, are useful in the evaluation of patients with autoimmune disorders. It is a rapid and sensitive method for simultaneous detection of 13 ANA autoantibodies and it displayed overall comparability to other traditional methods.

Recently, the Bioplex 2200 system has been installed in the Immunology Section of the Hamilton Regional Laboratory Medicine Program. Comparison and validation studies of this technology are now completed. Effective September 2008, we are offering ANA screening based on investigations of 13 autoantibodies simultaneously {dsDNA, Chromatin, Ribosomal protein, SS-A (52 and 60), SS-B, Sm, Sm/RNP, RNP(A and 68), Scl-70, Jo-1 and Centromere B}. ANA screen will be reported negative if the results of the tests for all the above-mentioned autoantibodies are negative (below cut-off value). ANA screen will be reported positive if any of the 13 autoantibodies investigated is positive (above cut-off level), and this report will show the actual levels of individual analytes. The positive result will include comments on possible disease association generated by MDSS. IIF testing for ANA can still be performed after discussion with the department.

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References: