

Line Immunoassays for Autoimmune Diagnostics

A short review of the technology, quality assessment, relevance of the platform and the possible issues of these new assays

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Autoimmune Diseases represents a fairly heterogeneous group of diseases with the common factor being a loss of tolerance to self. Associated with the Indian Diagnostics industry for several years, the author believes it would not be incorrect for him to judge autoimmunity as a somewhat neglected field on the Indian Medical scene. The overall awareness across Clinicians is limited on its diagnosis as well as on the treatment for such disorders.

Several reasons have been cited for this situation, issues including lack of financial resources of the patients, awareness among patients as well as clinicians, high cost of disease management, lack of specialists, over burdened medical system and above all, **the impracticality of routine diagnosis due to lack of samples and high costs of diagnosis**. While each of the above points can be debated upon, this paper is an attempt to explore whether the new Line Immunoassay (LIA) technology for autoimmune diagnosis through Immunoblotting can be the solution.

We explore here the following questions:

1. What is a Line Immunoassay (LIA)?
2. How is the quality of Line Immunoassay compared to other methods?
3. How relevant is this platform for routine diagnosis, confirmatory diagnosis and profiling for autoimmune diseases?
4. What are the possible issues that need to be kept in mind for LIA?

The Technology

The Nuclear/cytosolic antigens are applied as lines on a nitrocellulose membrane. The antigens may be in the native form, synthetic peptides or recombinant. The nitrocellulose membrane is blocked to prevent unspecific reactions. During the incubation process, the autoantibodies in patient serum will bind to these antigen lines on the membrane. For the detection of these bound antibodies, a labeled antibody directed against Human IgG is used. After addition of the substrate, the bound antibodies are visualized as lines. A stopping solution is then applied and the colour of the lines will change.

The different band locations correspond to different antigen coatings. Each visible band needs to be compared for intensity against a cut off control band. If the band intensity is higher than that of the cutoff control band, the result is interpreted as positive. ***The experienced users will find the principle quite similar to an Enzyme Immunoassay (ELISA). The key difference here is that the solid phase is a nitrocellulose membrane instead of the microtiter wells.***

The Quality Concerns

As with every new technology, the first and foremost question in medical diagnostics comes to 'Quality'. Quality can be defined on the basis of several parameters, important ones being the Sensitivity and Specificity. Here multiple studies have been performed globally. While some studies concentrated on ENA Antibodies, others looked at detection of specific autoantibodies using LIA. Eissfeller, Sticherling, Scholz, Hennig, Luttich and colleagues **reported Line Immunoassays to be sensitive as well as specific for Extractable Nuclear Antigens.**¹ Damoiseaux and colleagues compared the Line Immunoassay technique to Counter Immunoelectrophoresis (CIE), Enzyme Linked Immunosorbent assay (ELISA), fluorescent-enzyme immunoassay (FEIA) finding **Line Immunoassays to be better than CIE and similar to ELISA and FEIA.** They went on to conclude that **LIA is suitable for routine diagnosis of autoantibodies.**² Similarly, Prince and Hogrefe as well as other groups concluded that **Line Immunoassays yield results comparable to those of EIAs.**^{3,6} In a study specifically designed for assessing the usefulness of LIA for SSA and SSB autoantibody detection, Yalaoui and colleagues concluded that **Immunoblot based on Hela Cell can be used as a suitable alternate method.**⁴ López-Longo and colleagues went on to conclude that **LIA surpassed the performance of other assays** for Sm, Scl-70, SSB, Jo-1 (**Sensitivity 100%, Specificity 94-100%**) and was similarly effective for other autoantibodies.⁵

There is hence little doubt that Line Immunoassays are a sensitive as well as specific method for detection of various autoantibodies. They are similar in their sensitivity and specificity to ELISA.

Relevance of this New Platform

The current issues in autoimmunity in India include not just awareness but also a lack of testing capabilities at most diagnostic centers. While fluorescence is performed at only a few referral labs and large medical hospitals, even ELISA testing is not commonly performed in most laboratories. The reasons include minimal workload, lack of cost effectiveness due to run specific calibrations, large kit size of 96 Tests, absence of ELISA reader, inability to invest on kits for profiling etc.

The LIA in a large way is able to solve the above problems.

1. LIA allows profiling for a single patient – Through simultaneous detection of various autoantibodies, the Line immunoassay is possibly the best profiling platform available. Even in case of overlapping syndromes, the multiple antibodies can be simultaneously detected.
2. LIA does not require run specific controls and calibrators. As a result even a single test can be run without any additional costs. This means that laboratories no longer need to collect samples and run them together on designated days of the week.

3. LIA requires minimal training and laboratory equipment. As the reading is visual, any laboratory with basic skilled staff can efficiently perform the tests.
4. Cost Effectiveness: López-Longo and colleagues demonstrated that the cost of running a LIA is lesser than ELISA⁵ as several parameters can be simultaneously detected using a single strip. This is a significant cost saving especially when the workload is not very high.

LIA hence appears to be an excellent new platform for autoimmune testing, especially in the current Indian Scenario. **It is sensitive, specific, easy to use¹, cost effective⁶, requires minimal equipment and provides reproducible results.** For tests such as ANA, it can be used for profiling when an ANA is positive, or even directly as a first test when a patient is suspected of a systemic autoimmune disease such as Lupus, Scleroderma, Sjogren's disorder etc. On the other hand, profiles for Vasculitis and Autoimmune Gastroenterological diseases are now also being presented on Line Immunoassays. Here the LIA can be even used as a screening assay rather than doing several tests by ELISA. The laboratory needs to establish its own requirements and accordingly look at **LIA as a test for Screening, profiling or even confirmation.**

The Central Issues with LIA

Like with all technologies, even a Line Immunoassay does not answer all the requirements of the laboratory. **The biggest issue is that it is a qualitative assay** and though the band intensity relates to the concentration of antibodies, the test results cannot be quantified and reported. While for many autoantibodies, qualitative results suffice, for many such results are not sufficient, especially for monitoring the disease status. The test hence may not be useful for patient monitoring post detection. Most test panels are designed keeping this fact in mind.

The second issue which has been cited is the fact that it is a manual test which is **difficult to automate**. As a result large sample sizes cannot be simultaneously run. Also, there are still some tests which are not possible to run on a line immunoassay and have to be run on IFA or ELISA only.

There are new equipments being validated and made available which have the potential to solve both the above problems for quantification as well as automated processing of LIA. Till then the laboratories will have to suffice with the existing platform.

LIA has hence been shown as a sensitive as well as specific test for simultaneous detection of multiple antibodies. Its applications include screening, profiling as well as confirmation of results. Overall LIAs seems to offer an excellent option for autoimmune testing and have the potential to become an ideal platform for qualitative assessment of the autoantibodies.

An attempt has been made to provide the scientifically updated and impartial information. The author can be contacted for any clarifications, further literature, comments, queries and feedback regarding this article as well as autoimmune diagnostics in general at the following email address: prateek@paramlife.com

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