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Letter to the Editor

Luciferase Immunoprecipitation System Assay, a Rapid, Simple, Quantitative, and Highly Sensitive Antibody Detection for Parasitic Diseases

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Dear Editor-in-Chief

Studying immunoglobulin responses by serological techniques is imperative for evaluating antibody titer values during the course of some parasitic infections. Therefore, the presence of a rapid, high-throughput, cost-effective and safe method, which can minimize both false positive-and false negative-cases, is beneficial for early diagnosis and accurate treatment of patient. Accordingly, the aim of this letter is to highlight the potential for a novel nonradioactive solution phase immunoassay called luciferase immunoprecipitation system (LIPS).

Some restriction of solid phase arrays employing bacterial expressed proteins is producing high backgrounds due to presenting bacterial contaminants, thus producing highly purified antigens is time-consuming and labor intensive. In addition, proteins expressed via bacteria as recombinant antigens do not represent either mammalian or disease specific posttranslational modification. On the other hand, coating recombinant proteins on nitrocellulose membranes or slides do not allow proper exposure of the conformational epitopes and having limited dynamic range of detection (1, 2).

To circumvent these problems we have developed a novel solution phase assay designated luciferase immunoprecipitation system (LIPS) (1). Luciferase immuoprecipitation system is a nonradioactive diagnostic approach applied for assessment humoral responses in which target antigen is fused to the enzyme

reporter renilla luciferase (Ruc) for generating Ruc-antigen fusion protein which is performed by designing and cloning appropriate plasmid expression vector in format of pREN2 or pREN3S (mammalian Ruc expression vector), then the recombinant plasmids are transferred to monkey kidney cells (Cos1 cells) or other cells (e.g., HEK293) for exerting-mammalian-specific posttranslational modifications. After lysing Cos1 cells, Ruc-antigen extracts are directly used in LIPS without purification. This process involves blending patient sera samples, tagged Ruc antigen together, then the admixture of Ruc-antigen-blending patient sera samples, and tagged Ruc antigen together, then the admixture of Ruc-antigen-antibody complex is loaded to a microtiter filter plate containing protein A/G beads to trap antibodies. Eventually, after the filter plate is rinsed out from unbound Ruc-antigen with wash buffer, coelenterazine substrate is added to emit light reported as light units by a luminometer (Fig. 1) (3, 4).

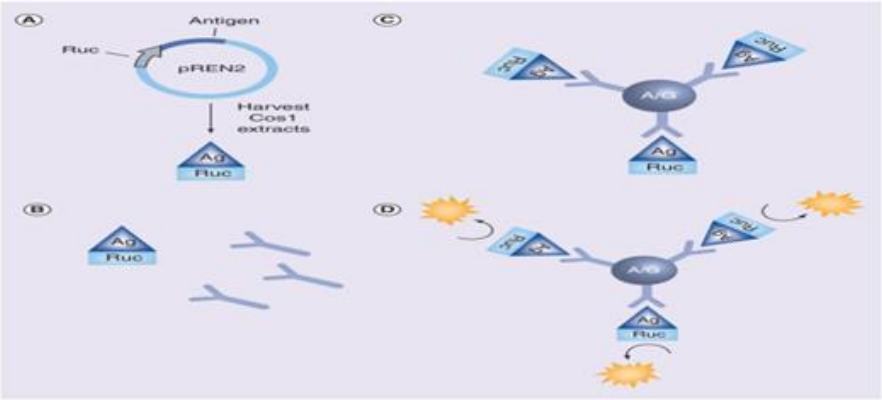

Fig. 1: Luciferase immunoprecipitation assay

In recent years, this approach has been successfully applied for profiling *Loa-loa*-specific antibody responses by using a Ruc-LISXP1 fusion. LISXP1-IgG4 based LIPS for identifying *Loa-loa* infected patients demonstrated increased sensitivity (100%) and specificity (100%) with a determinate cross-immunoreactivity with other filarial infections, afew *Onchocerca volvulus* and *Wucheria bancrofti*-infected patient sera. Unlile LIPS, ELISA-based assay measuring anti-LISXP1 showed rational sensitivity (67%) and specificity (99%). For diminishing cross-reaction, the incubation time are detracted by employing shortened format of 2.5 h LIPS, designated quick 15 min QLIPS (5). LIPS is highly remedial for simultaneously detecting antibodies to a synthesis of multiple Ruc-antigen in a few microliters of serum (1). This assay has shown markedly improved sensitivity (100%) and specificity (100%) compared to existing immunoassays for profiling *Strongyloides stercoralis*-specific antibody responses by using recombinant SsIR antigen in combination with NIE antigen (6). The multiple-antigen LIPS assay yields highly robust antibody titers for diagnosing of *Onchocerca volvulus* by using a panel of recombinant antigens including (Ov-FAR-1, Ov-API-1, Ov-MSA-1 and Ov-COI-1),in this case specificity and sensitivity increased up to 100% (7).The results suggest that LIPS has some merits such as, having high specificity and sensitivity with a liner dynamic range of detection (8), being safe and cost-effective, profiling antibodies rapidly and simply.

Accordingly, LIPS is extremely informative tool as an alternative to ELISA and RIA for monitoring antibody responses associated with variety of parasitic infectious agents (9).

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