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Luciferase Immunoprecipitation Systems for Measuring Antibodies in Autoimmune and Infectious Diseases

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Abstract

Antibody profiles have the potential to revolutionize personalized medicine by providing important information related to autoimmunity against self-proteins and exposure to infectious agents. One immunoassay technology, Luciferase Immunoprecipitation Systems (LIPS), harnesses light emitting recombinant proteins to generate robust, high quality antibody data often spanning a large dynamic range of detection. Here we describe the general format of LIPS and discuss studies using the technology to measure autoantibodies in several human autoimmune diseases including type I diabetes, Sjögren's syndrome, systemic lupus erythematosus, and immunodeficiencies secondary to anti-cytokine autoantibodies. We also describe the usefulness of evaluating antibodies against single or multiple antigens from infectious agents for diagnosis, pathogen discovery and for obtaining individual exposure profiles. These diverse findings support the notion that LIPS is a useful technology for generating antibody profiles for personalized diagnosis and monitoring of human health.

Keywords

antibodies; autoantibodies; autoimmunity; infections; Luciferase Immunoprecipitation Systems (LIPS); personalized medicine; pathogens; viruses

Introduction

Antibodies are key components of the immune system that are able to bind with great specificity to an extremely large variety of target molecules. In addition to their important function in the adaptive immune response, antibody testing represents a major tool for the

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diagnosis of many infectious agents including current and past exposures.[1] The detection of antibodies against self-proteins, autoantibodies, is also important for the diagnosis of a variety of autoimmune diseases. In some autoimmune diseases, autoantibodies are present before the onset of clinical symptoms, and for certain targets, autoantibodies can play a direct role in causing pathogenesis. Although the full spectrum of diagnostically useful antibodies are not currently known, generating comprehensive antibody profiles will likely represent an important next step in understanding human health. In this review, we describe the Luciferase Immunoprecipitation Systems (LIPS) antibody profiling technology and discuss its wide range of applications and the types of information that can be obtained from employing the technology. An in-depth discussion of other immunoassay technologies used to measure antibodies can be found in a recent review.[1]

LIPS technology

The clinical information provided by quantifying antibodies has long been recognized, but the most common immunoassay technologies such as Western blotting and ELISA, used to measure the amount of antibodies against particular targets, have a number of drawbacks including a limited ability to efficiently detect conformational epitopes, a limited dynamic range of detection, and high backgrounds frequently associated with using crude protein preparations or bacterial recombinant proteins.[2] In contrast, fluid-phase radioimmunoprecipitation assays employing radiolabeled antigens can overcome many of these limitations and are the method of choice for measuring autoantibodies in autoimmune diseases.[3] Despite the usefulness of the radioimmunoprecipitation assay, the requirement for radioisotopes limits its widespread application. An alternative to radioimmunoprecipitation assays for measuring antibodies is the fluid-phase Luciferase Immunoprecipitation Systems (LIPS) technology, which is based on luciferase-tagged antigens produced in mammalian cells.[1,2] With the LIPS technology, the gene encoding the 30 kDa luciferase, isolated from the soft coral *Renilla reniforms*, is typically used as the reporter because this light-producing enzyme has a highly linear output spanning over seven orders of magnitude. The construction of *Renilla* luciferase (Ruc) chimeric genes involves standard molecular techniques with mammalian expression vectors (e.g. pREN2) in which the antigen of interest is fused in-frame with Ruc.[4,5] A variety of recombinant protein targets can be employed in LIPS including full-length proteins, protein variants and fragments, and short peptides. Non-protein targets such as phospholipids, DNA, and RNA cannot be used in LIPS.

To initiate LIPS, plasmids encoding these light-emitting antigen fusions are first transfected into Cos1 mammalian cells (Fig 1). Since the antigen is directly tagged with luciferase, crude extracts are used without the need for time-consuming protein purification. Importantly, many of the crude extracts containing the Ruc-tagged antigens can be stored as frozen aliquots and can be thawed for use at a later time. For antibody testing, a defined amount of the Ruc-tagged recombinant protein based on light units (LU) is first incubated with each serum sample typically for one hour. In these assays, 1.0 microliter of serum is used, potentially allowing up to 1000 determinations to be made from 1 mL of serum or plasma. During this first incubation step, antibodies in serum, if present, bind to the target antigen fused to Ruc (Fig 1). The reaction mixture is then transferred for an additional hour

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to a filter plate containing antibody capturing reagents such as protein A/G beads or other secondary immunoglobulin-immobilized beads. While these beads can bind both free immunoglobulins and antibodies bound to the Ruc-tagged antigen, free unbound luciferase-tagged antigen is removed from the microtiter filter plate by multiple washing steps. Next, the relative amount of antibody bound to the *Ruc*-tagged antigen can be determined by measuring the light produced when adding coelenterazine, the substrate for Ruc (Fig 1). It should be noted that a variety of LIPS formats can be performed to collect highly quantitative antibody data including single tube assays[5], 96-well plates[4], rapid tests[6,7], arrays[8], and even a microfluidic device. [9] The time required to perform LIPS testing is under 2.5 hours and typically faster than ELISA and Western blotting. Although there are currently no commercial products available for specific LIPS tests, the LIPS vectors used to generate light-emitting proteins are available upon request (PDB).

Detection and analysis of autoantibodies by LIPS in autoimmune conditions

Autoimmune diseases are quiet common conditions and are associated with significant morbidity and mortality costs. For many autoimmune diseases, genetic information offers limited diagnostic or predictive clinical value because these complex diseases are not caused by single genetic alterations, but rather involve multiple weakly associated gene polymorphisms interacting with various environmental factors.[10] On the other hand, autoantibody detection in autoimmune conditions represents an important tool for personalized care providing information for diagnosis, monitoring and even disease prediction. Here we describe the application of LIPS for measuring autoantibodies in wide range of autoimmune studies yielding improved diagnostic performance and/or new information (Table I).

In type I diabetes (T1D), an autoimmune disease involving the destruction of insulin-producing pancreatic beta cells, several different autoantibodies have been identified including insulin, GAD65, IA2, IA2-β and Znt8. While the radioimmunoprecipitation assay, a fluid-phase immunoassay, is the gold standard for detecting T1D-associated autoantibodies[3], LIPS represents a promising non-radioactive alternative. Comparative studies have shown that both LIPS and RIP have similar sensitivity and specificity for detecting autoantibodies against several of the major T1D autoantigen.[11,12] For example, the detection of anti-IA2 autoantibodies in T1D patients by LIPS demonstrated 85% sensitivity and 100% specificity and autoantibody values obtained correlated well radioimmun oprecipitation assay.[12] In these studies, the dynamic range of detection for the LIPS assays was larger than the radioimmunoprecipitation assay and spanned 10^3-10^5 LU. Several other investigators have successfully utilized LIPS as a non-radioactive alternative for measuring autoantibodies in T1D. [13-17] Autoantibodies against the relatively newly identified autoantigen, PAA, were shown by LIPS to be more prevalent in T1D patients harboring autoantibodies against multiple autoantigens.[13] Lampasona et al. detected high levels of robust autoantibodies by LIPS against harmonin and villin in a majority of patients with the autoimmune condition Immunodysregulation, Polyendocrinopathy, Enteropathy X-linked syndrome (IPEX), but found no evidence of these autoantibodies in T1D.[14] It is also important to point out that other immunoassays including ELISA and protein array are often inadequate for detecting autoantibodies in T1D and other autoimmune conditions.[3] The lack

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of diagnostic utility of these solid phase assays is exemplified by a recent study showing that a protein array technology was unable to detect *any* significant autoantibody responses against the two major known autoantigens, GAD65 and IA2, which were detectable by LIPS in the same T1D patient serum samples.[16]5

Acquiring autoantibody profiles against multiple autoantigen targets in T1D is highly desirable because studies have shown that the number of different islet autoantibodies present in a given individual has the greatest predictive value for determining which children will go on to develop diabetes.[18] In addition, T1D patients can also show evidence of other comorbid autoimmune conditions that can be detected by the presence of autoantibodies associated with these conditions. Due to the modular nature of the assay and the ability of using crude extracts without purification, LIPS is ideal for testing multiple autoantigens. Additionally, the assay development time is generally much shorter than other solid-phase immunoassays that employ native or recombinant proteins. This is because the development of the different light emitting protein detectors for LIPS simply involves cloning and transfection, in which the extracts can generally be used in a standard format without optimization. In one investigation autoantibodies against nine targets were measured in T1D patients.[19] Not only did the T1D patients show a high frequency of T1D associated autoantibodies, but approximately 50% of the T1D patients had autoantibody responses against at least one other extrapancreatic target including the thyroid peroxidase associated with Hashimoto's thyroiditis, TGM2 associated with celiac disease and the gastric ATPase associated with autoimmune gastritis. Although the clinical data for this cohort was not available for analysis, this study demonstrates the possibility of using LIPS to identify patient subgroups with different clinical symptoms or for further examining potential genetic associations.

In addition to the high frequency of GAD65 autoantibodies found in T1D patients, autoantibodies against GAD65 can also be detected in several neurological diseases including Stiff Person syndrome and ataxia.[20] In Stiff Person syndrome, LIPS detected highly robust levels of GAD65 autoantibodies and achieved diagnostic accuracy of 100% sensitivity and 100% specificity.[21] In another study, LIPS was also employed to screen a cohort of psychiatric patients for autoantibodies.[22] High levels of GAD65 autoantibodies were detected in a female patient with major depressive disorder who showed signs of psychomotor slowing, a clinical condition characterized by slow movement of her extremities. Elevated GAD65 autoantibodies were also highly detectable in cerebrospinal fluid of the patient and the autoantibody levels correlated over time with clinical severity suggesting that CNS autoimmunity might be responsible for psychomotor impairment. These findings support the idea of uncovering unrecognized autoimmune pathogenesis in human disease by autoantibody profiling.

Sjögren's syndrome (SS) is an autoimmune disease characterized by autoimmune attack on the salivary and lacrimal glands leading to decreased saliva and tear production, respectively.[23] One objective criterion for the diagnosis of SS involves measuring autoantibodies against SSB (La) and SA (Ro52 and Ro60). LIPS showed 75% sensitivity for the detection of La autoantibodies compared to 45% sensitivity for an established ELISA in a small cohort of SS patients and healthy controls.[24] For SSA, LIPS separately detected

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autoantibodies against Ro52 and Ro60 and showed similar diagnostic performance to the ELISA which measured them together. Measuring autoantibodies against other targets also revealed that some patients also had significant autoantibodies associated with other autoimmune conditions. For example, 16% of SS patients had autoantibodies against thyroid peroxidase, an autoantigen associated with Hashimoto's thyroiditis. Similarly, 14% and 12% of the SS patients had autoantibodies against gastric ATPase and aquaporin-4, respectively, representing potential autoimmune attack on the stomach and nervous system.[24] Compared to the standard 2.5 hour testing format, a rapid 15 minute LIPS test showed promise for detecting anti-Ro52 serum autoantibodies for the diagnosis of SS.[6] The highly robust LIPS assay can be used for non-invasive testing by using saliva instead of a serum as the clinical sample. Using saliva, Ro60 autoantibodies showed 75% sensitivity and 96% specificity for the diagnosis of SS and correlated with serum levels of Ro60 autoantibody.[25] The ability to examine antibodies in saliva has additional applications for non-invasively studying humoral responses against infectious agents.

Systemic lupus erythematosus (SLE) represents a relatively common autoimmune disease characterized by inflammation and chronic immune attack on various cells and tissues.[23] The detection of autoantibodies in SLE is critical for diagnosis, disease monitoring and can predict disease onset.[26,27] Ching et al. used LIPS to detect autoantibodies against a panel of autoantigens revealing that most SLE patients had either a cluster I or cluster II antibody phenotype.[28] Patients with a cluster I phenotype showed enriched autoantibodies against Sm, U1-RNP-A1, and U1-70K RNP RNA-binding proteins, while patients with a cluster II phenotype had enriched autoantibodies against Ro52, Ro60, and La. The exact method for analyzing the LIPS autoantibody data from the SLE patients simply involved comparing the responses against Sm, U1-RNP-A1, and U1-70K RNP vs. Ro52, Ro60, and La. In contrast, a previous study based on ELISA immunoassay results identified the two similar autoantibody clusters in SLE utilizing the relatively complex *K*-means clustering algorithm.[29] Overall, these findings highlight the ease of antibody analysis when robust and highly quantitative values are generated by the LIPS technology. Another unique feature of LIPS is the possibility of employing antigen mixtures for achieving high diagnostic performance from a single test.[28,30,31] The mixture format is based on fact that the protein the A/G beads used in the assay binds many different immunoglobulins present in serum. When incubated with multiple light emitting antigens, these bound immunoglobulins can interact with multiple detectors yielding an overall signal similar to performing these tests separately (Fig. 1). In the case of the diagnosis of lupus, a single LIPS mixture test incorporating seven antigens (Sm, U1-RNP-A1, U1-70K RNP, Ro52, Ro60, and La) combined with one microliter of serum matched the sensitivity and specificity of performing these seven individual LIPS tests separately. [28] Since this approach does not provide information about the specific target proteins that are immunoreactive in seropositive samples, if needed, additional follow-up testing of individual antigens would need to be performed. Nevertheless, this LIPS mixture approach saves time and resources and has also been used to simplify the diagnosis of several infectious agents.[30,31]

Besides autoantibodies as biomarkers, pathogenic autoantibodies can interfere with normal processes and directly cause human disease. Pathogenic autoantibodies can be generated

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against cytokines, which are secreted molecules that play critical roles in regulating the immune response to infection. LIPS detected high levels of anti-cytokine autoantibodies in several diseases including in certain patients with acute respiratory distress syndrome and sepsis[32], SLE [28], and in opportunistic infections secondary to anti-cytokine autoantibodies.[33,34] In thymoma patients exhibiting opportunistic infections such as mucocandidiasis and disseminated varicella zoster virus, LIPS identified multiple anti-cytokine autoantibodies as the likely culprits involved in pathogenesis.[34] Although LIPS detected known elevated levels of anti-interferon-α autoantibodies, high levels of autoantibodies against IL-12 p35, IL12-p40 and IL-17 correlated better with the presence of opportunistic infections in these patients. The levels of autoantibodies detected by LIPS against these cytokines also matched functional assays for STAT-4 neutralization experiments. Lastly, this study also highlighted how LIPS can be used for autoantigen discovery. In this study, systematic LIPS screening of 39 different candidate cytokines resulted in the discovery of two patients with previously undescribed autoantibodies against the BAFF cytokine.[34]

Due to the ability to screen many different antigens in parallel, LIPS has been used to analyze a cohort of disseminated nontuberculous mycobacterial infections (dNTM) patients to understand the full spectrum of anti-cytokine autoantibodies. [33] dNTM patients are severely immunosuppressed and harbor infections with numerous rapid and slow growing mycobacteria. From analyzing autoantibodies against 41 cytokine and other immune targets, approximately 90% of the dNTM patients demonstrated interferon-γ autoantibodies, which routinely were 1000 times higher than the levels found in the controls. The levels of interferon-γ autoantibodies detected by LIPS in the dNTM patient serum samples also tracked their ability to neutralize downstream signaling activity as detected by *in vitro* assays. One dNTM patient did not have interferon-γ autoantibodies, but showed high levels of anti-GMCSF autoantibodies.[33] Remarkably, no significant autoantibody seropositivity was detected against the other cytokines. These results suggest that the LIPS technology is useful for identifying pathogenic autoantibodies against cytokines and potentially other extracellular targets.

Since the full spectrum of human diseases showing autoantibodies is not known, there are likely to be other diseases that may have an unrecognized autoimmune component. Two such diseases fitting this category are acute respiratory distress syndrome and sepsis, conditions characterized by intense immune activation leading to organ failure. LIPS not only detected evidence of robust autoantibodies against several autoantigens including cytokines and known autoantigens, but demonstrated that there was rapid induction of some autoantibodies in only a few days and suggests that ongoing inflammation may mediate the break in tolerance to self-proteins.[32] Collectively, the results presented highlight the possibility of using LIPS to detect new biomarkers for many other diseases including conditions that are not classically thought to involve autoimmune responses.

Diagnosis and Monitoring Antibody Responses to Infectious Agents

Antibody testing represents a major tool for the diagnosis of many infectious agents and provides insight into current and past exposure and even response to vaccines. Liquid phase

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immunoassays such as radioimmunoprecipitation assays have not been generally employed to measure antibodies against infectious agents due to the requirement for radioactive labeling. With the recent development of the LIPS, this liquid phase immunoassay has been used to interrogate antibodies against a wide range of different infectious agents including fungal, filarial, bacterial, and viral agents.[1,2] Below we describe these studies and discuss how LIPS has provided new information for detection antibodies against infectious agents (Table II).

One key advantage of LIPS is the highly robust antibody levels that are often seen against multiple proteins from a given infectious agent, which is helpful in distinguishing infected patients from uninfected controls. For several infectious agents, side-by-side comparison has shown improved diagnostic potential of LIPS over classic ELISA tests. For example, LIPS showed higher sensitivity compared to ELISA and several other immunoassays for the detection of *Strongyloides stercoralis*[35,36], *Onchocerciasis*[31] and *Loa loa* infection.[7] For other agents, the diagnostic performance of LIPS has been comparable to ELISA tests but has shown additional useful features.[30,37,38] For example, a LIPS test for *Borrelia burgdorferi*, the bacterial cause of Lyme disease, also matched the diagnostic performance of an established Lyme ELISA test [37] and has been used to exclude a major role of Lyme disease in the pathogenesis of autism.[39] A LIPS test for a common herpes virus infection, Varicella-Zoster virus (VZV), was able to distinguish vaccinated from unvaccinated subjects and matched the gold standard fluorescent antibody to membrane antigen (FAMA) test. [40] For the Epstein Bar virus (EBV) herpesvirus, a LIPS test detecting antibodies to EBV gp350 correlated with antibody neutralization assays, which could be useful for measuring responses to EBV vaccines.[41]

The ability of using crude lysates from transfected cells without protein purification also makes LIPS a practical approach to measure antibodies against different proteins from a given infectious agent including the whole proteome of HIV [8,42] and partial proteomes of HTLV-I, HCV [8,43,44], and EBV [8]. This approach is also useful for screening and identifying new antigenic proteins from infectious agents. For example, a screen of sixteen antigens from *Wuchereria bancrofti* identified WB123 as a highly informative antigen for the diagnosis of this filarial infection.[45] Similarly, screening of 20 proteins from Kaposi-associated herpesvirus (KSHV/HHV-8) identified robust antibodies against v-cyclin in 75% of Kaposi sarcoma patients. [30] Interestingly, antibodies against v-cyclin were not detected in several solid phase formats including Western blot [46] and protein array [47] consistent with the better detection of conformational antibodies by the liquid phase immunoassays compared to solid phase assays.[3] A LIPS mixture assay for four KSHV antigenic targets efficiently detected infection and matched the sensitivity and specificity of performing two separate ELISA tests.[30]

Owing to the ability of LIPS to detect antibodies against multiple antigens from different viruses, unique antibody profiles were identified in patient subsets including patients infected with HTLV-I [48], EBV [49], KSHV [50], and HIV.[51] For example, patients with HAM/TSP a debilitating neurologic disease caused by HTLV-I viral infection showed high levels of anti-envelope HTLV-I antibodies compared to asymptomatic infected individuals or patients with HTLV-I associated lymphoma. [48,52] In chronic active EBV patients, anti-

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EBV antibody profiling showed higher antibody levels against several lytic EBV antigens compared to healthy controls, which is consistent with increased EBV replication in these patients.[49] In two KSHV-associated diseases, the relative antibody levels against lytic vs. latent viral antigens was markedly higher in Multicentric Castlemen's disease compared to patients with Kaposi Sarcoma.[50] The different antibody patterns seen in the patient subgroups likely reflect altered protein expression and/or immune recognition of these infection agents.

LIPS has also provided simple biomarkers for studying clinical subsets of HIV patients. Mendozza et al. analyzed a cohort of elite HIV controllers, patients who showed exquisite control over HIV infection, and found a novel low antibody response signature against reverse transcriptase, protease, and integrase consistent with the possibility that these patients had low levels of replicating HIV virus.[51] In a subsequent HIV study, humoral responses were examined by LIPS against nine HIV proteins in patients with low HIV viral load including elite controllers, anti-retroviral treated patients and in the Berlin patient, the first patient cured.[53] A key finding was that the Berlin patient had undetectable antibodies against p24 and five other HIV proteins, but still had weak HIV antibody responses against tat, gp41, and reverse transcriptase. In another study of HCV-HIV coinfected patients, a novel HCV antibody biomarker profile was identified by LIPS, which correlated with response to treatment with interferon alpha and ribavirin.[43] Overall, these results with HIV and HCV suggest that these quantitative antibody profiles generated by LIPS could be used to monitor therapy.

In spite of the identification of novel infectious agents by nucleic acid amplification and DNA sequencing approaches, it is important to point out that the full spectrum of infectious agents and their impact on health remains largely incomplete.[54] Since detection of specific antibody responses can provide *in vivo* evidence of infection, the robust antibody responses obtained by LIPS can be used as another tool for pathogen discovery. For example, numerous astroviruses have been identified by metagenomic sequencing of human stool [55,56], but the *in vivo* relevance of these agents is unknown. LIPS testing of one of the new astroviruses, HMOAstV-C, revealed significant antibodies to the capsid of this virus in humans, but not in several animal species including pigs and rabbits. Further analysis of samples from children revealed that approximately 20% of one-year-old children and approximately 65% of adults showed antibodies against HMOAstV-C.[57] These findings support the incorporation of high quality serologic LIPS data for accelerating the discovery of new pathogens obtained from nucleic acid discovery efforts.

LIPS can also be helpful for identifying animal reservoirs of human-related viruses. In one study LIPS was used to identify the natural reservoir of HCV-like viruses in non-primates.[58] The area of research initially started by Kapoor et al. who discovered the first non-primate homolog of HCV called canine hepativirus in two dogs.[59] No serology was performed at the time and the development of a subsequent LIPS test against the capsid and helicase regions of canine hepativirus failed to detect antibodies in over 120 canine serum samples suggesting infection by this virus may be a rare event in dogs. However, additional testing of a variety of other animals revealed that approximately 40% of horses showed robust antibody responses against the helicase. DNA analysis of the seropositive horses identified 8

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new genetically distinct, non-human primate hepatitis-like viruses (designated NHPV) in these equine samples and establish horses as a major reservoir of HCV-like viruses.[58] More recently, LIPS was used to investigate the animal reservoir of Middle East respiratory syndrome virus (MERS), a potential lethal viral infection in humans occurring in Saudi Arabia.[60] While no serologic evidence for MERS infection was observed in sheep and goats, camels were identified as source of MERS. These results highlight how LIPS can be used to discover novel pathogens in animals which can cause zoonotic infections in humans.

Increasing evidence suggests that the complicated interaction of our bodies with microbial agents and even exposure against many infectious agents that do not cause overt disease may influence human health. One important opportunity for personalized health profiles will be to define individual exposure profiles to multiple infectious agents. As a proof of concept, LIPS was employed to measure antibodies against thirteen common infectious agents in three different chronic diseases, patients with HIV, interferon-γ autoantibodies, and Sjögren's syndrome.[61] Rather than focus on antibody responses to any one individual infectious agents, the cumulative antibody data was modeled by principal component analysis. For both HIV patients and patients with high levels of autoantibodies against interferon-γ, a distinct antibody profile was observed compared to healthy control subjects. Moreover, there was a noticeable difference between these profiles highlighting the fact that each disease perturbs different specific immune pathways. [61] In contrast, the Sjögren's syndrome cohort did not reveal an informative profile suggesting that these infectious agents might be less relevant for this disease.[61] Based on these promising findings, it is likely that the incorporation of additional infectious agent targets into the panel might make this approach even more informative. Finally, the ability to profile so many different infectious agents in a single format presents a powerful tool for diagnosis and personalized medicine and might be configured into a novel immune readout of overall immune health.

Conclusion

In this review we have discussed how LIPS, a liquid phase immunoassay, provides important information for diagnosis, monitoring, and insight into disease pathogenesis. These many diverse studies provide a platform for more extensive interrogation of antibody profiles in human disease. Five major attributes make LIPS a highly desirable technology for studying antibodies: (1) an assay that does not require radioactivity, (2) the possibility of stably storing the fusion antigen extracts for facile testing on demand, (3) the high signal to noise ratio, and (4) the wide dynamic range of antibody detection. Since LIPS requires small amounts of serum, hundreds of targets could theoretically be profiled with the serum obtained from several milliliters of processed blood. Besides testing one protein at a time, LIPS arrays offer an alternative format for simultaneously multiple antibodies, in which different light emitting protein targets are tested in individual wells of a 96-well plate with control and test serum samples. As proof of concept, LIPS arrays detected highly robust immunoreactivity against multiple proteins derived from HCV, HIV and EBV proteomes in infected subjects compared to uninfected controls.[8] Based on these results, LIPS arrays could be used as a discovery tool for simultaneously measuring antibody levels against candidate target proteins in autoimmune and infectious diseases.

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It is expected that in the coming years, antibody profiles generated by LIPS will continue to provide important information related to pathogenesis and diagnosis. One strategy for predictive medicine involves monitoring antibodies longitudinally over time to many different targets. Along these lines, it is conceivable that a library of known autoantigen targets used in LIPS could prove sufficient for the broad diagnosis of many common autoimmune disorders. Since it is currently known that autoantibodies are present years before clinical onset of several autoimmune conditions including T1D [62], SLE [26], and SS [63] and it is possible that LIPS and other assays, along with clinical information including association with family members and presence of risk genes, could be efficiently used to identify individuals at high risk for developing these and other conditions including cancer and other age-related neurological diseases. In addition, the approach of using LIPS serology to profile antibodies against multiple infectious agents could be used to identify novel agents that might contribute to inflammation and impact the likelihood of developing certain diseases. Most importantly, these antibody profiles might be used for early detection thereby identifying patients before clinical onset, which may allow a change in lifestyle or treatment options that might delay or further prevent disease onset or progression.

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All authors have read the journal's policy on disclosure of potential conflicts of interest. One of the authors (P.D.B.) has three patent applications submitted regarding the use of LIPS for detecting antibodies against KSHV/HHV-8, *Wucheria Bancrofti* and Lyme disease. The two other authors have no potential conflicts of interest to declare. All authors have also read the journal's authorship agreement and that the manuscript has been reviewed by and approved by all named authors. No editorial support was used in the preparation of the manuscript. This work was supported by the intramural research program of the National Institute of Dental and Craniofacial Research, NIH.

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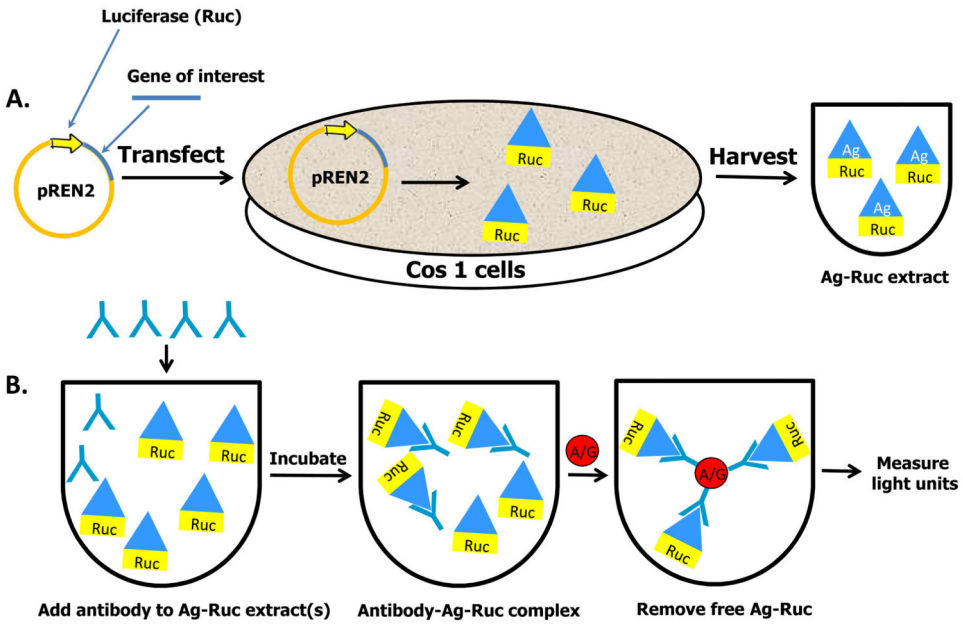

Figure 1.

Schematic of the general steps involved in LIPS. (A) The DNA sequence of the antigen of interest is genetically fused to the C-terminus of *Renilla* luciferase (Ruc). These recombinant plasmids are then used to transfect Cos1 cells and cell lysate is harvested 48 hours later without purification. (B) Aliquots of a single extract for a Ruc-antigen or a mixture of multiple extracts for different Ruc-antigens are then incubated with serum samples. The antibody complexes are then captured by protein A/G beads and the unbound luciferase tagged antigen is washed away. The amount of specific antibodies present is then determined by the amount of bound antigen present by adding luciferase substrate.

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Table I

LIPS for Autoantibody Detection

Disease/Infection	Examples of Important Findings Made by LIPS
Type I diabetes	• LIPS diagnostic performance matched gold standard radioactive binding assayfor detection of IA-2, IA2-β and GAD-65 autoantibodies.[11,12] • Detected high frequency of autoantibodies against other autoimmune targets inT1D patients for potentially identifying patient subsets.[19]
IPEX	• Identified autoantibodies against harmonin and villin as diagnostic markers in children with Immunodysregulation, Polyendocrinopathy, Enteropathy X-linked syndrome (IPEX) syndrome.[14]
Stiff-person syndrome	• LIPS showed 75% sensitivity for detection of GAD65 for diagnosis of Stiff-person syndrome.[21] Identified a psychiatric patient with psychomotor slowing with high GAD65 autoantibodies in serum and cerebrospinal fluid.[22]
Sjögren's syndrome	• LIPS showed 75% sensitivity for detection of SSB autoantibodies compared to45% sensitivity for ELISA.[24] • Detected 16% frequency of autoantibodies against gastric ATPase in Sjögren'ssyndrome patients.[24] • Sensitive detection of Ro60 and Ro52 autoantibodies for diagnosis in the salivaof Sjögren's patients.[25]
SLE	• Found 95% of SLE patients to contain one of two types of autoantibody clusters.[28]
Thymoma patients	• Identified a subset of thymoma patients with multiple anti-cytokineautoantibodies, which correlated with opportunistic infections in patients.[34] • Relative levels of anti-cytokine autoantibodies detected by LIPS correlated with*in vitro* neutralizing activity.[34]
dNTM	• Patients with disseminated non-tuberculous mycobacterial infection (dNTM)show high frequency of high titer IFN-γ autoantibodies.[33] • Autoantibodies against over thirty other cytokine in dNTM were not detected.[33]
ARDS and Sepsis	• Observed the rapid induction of autoantibodies in patients with acute respiratory distress syndrome (ARDS) and sepsis.[32]

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Table II
LIPS for Antibody Detection of Infectious Agents

Disease/Infection	Examples of Important Findings Made by LIPS
Strongyloides stercoralis	• LIPS showed improved serologic test compared to ELISA for *Strongyloidesstercoralis*.[35,36] • Demonstrated utility of evaluating antibody responses for monitoring therapyof *Strongyloides stercoralis*.[35]
Onchocerca*volvulus*	• Improved serologic testing compared to ELISA for *Onchocerciasis*.[31]
Loa loa	• Improved serologic testing compared to ELISA for *Loa loa* infection.[7] • Developed high performance rapid test for *Loa loa* antibodies.[7]
EBV	• Discovered a unique antibody profile in patients with chronic active EBV.[49] • Demonstrated anti-gp350 EBV antibodies detected by LIPS correlated withneutralizing anti-EBV antibodies.[41]
HIV	• Evaluated antibodies against the HIV proteome using defined recombinant proteins.[42] • Discovered unique antibody signature in subset of elite HIV controllers.[51] • Obtained evidence for near absence of anti-HIV antibodies in the first personcured, the Berlin patient.[53] • Distinguished HIV patients from blood donor controls based on broadantibody profiles against multiple common infectious agents.[61]
HTLV-I	• Analyzed antibodies to full HTLV-I proteome and discovered high levels of anti-envelope antibodies in patients with HAM/TSP compared to asymptomatic or patients with associated lymphoma.[48,52]
HCV	• Identified an antibody profile that correlated with response to treatment in HCV-HIV infected patients.[43]
Wuchereria bancrofti	• From screening 16 *Wuchereria bancrofti* proteins for antibodies, identified Wb123 as an early and specific marker for infection.[45,64]
KSHV	• Identified v-cyclin as a new robust serologic marker for KSHV infection. [30] • Found elevated antibody responses against lytic vs. latent viral proteins inMulticentric Castlemen's disease compared to Kaposi sarcoma patients.[50]
NPHV	• LIPS screening of different animals identified a novel non-primate HCV-like virus (NPHV) in horses.[58]
MERS	• Provided serological evidence that camels have harbored Middle East respiratory virus (MERS) for over 20 years.[60]