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Research Article

Immuno-diagnosis of Some Microbial Antigens and Antibodies Using Dissociated Immune Complexes and Serum: A Comparative Study

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ABSTRACT

The principles of Immunological methodologies have always based on the specificity and sensitivity of the antibody to its corresponding antigen. This study was designed to compare the specificity and sensitivity of antigens and antibodies in unprocessed serum and serum treated with Polyethylene glycol for precipitation and dissociation of immune complexes. One hundred (100) human sera samples were randomly collected. The samples were duplicated and one was treated with polyethelene glycol 6000 and buffer to precipitate and dissociate immune complexes. The end product was tagged immune solution. This solution was assayed along side its unprocessed serum concurrently to detect HIV, HCV, HBV, *Plasmodium falciparum* and *Salmonella typhi* and *Salmonella paratyphi* antibodies respectively using Immuno-chromatographic assay method. Out of the 100 sera samples analyzed, the serum samples showed positive to 10 HIV test, 21 for *Plasmodium falciparum*, 2 for HCV, 0 for HBV. Immune solution showed positive for 13 HIV, 26 *Plasmodium falciparum*, 5 HCV and 2 HBV. Antigens and antibodies present in dissociated form in immune solution was found to be more suitable for immunological assays. With this development, the preference of immune solution to serum for serological tests is established. Before any epidemiological survey is concluded for a particular disease condition in a locality, it is suggested that the serum should be used along side its processed immune solution.

Key Words: Serum, Immune Complex, Immuno-diagnosis, Dissociation

INTRODUCTION

Polyethylene Glycol (PEG), is a laboratory reagent that has been widely used in the antigen antibody precipitation, such as in analysis of circulating immune complexes, and in production of monoclonal and polyclonal antibodies (Mark *et al.,* 2009). Circulating immune complexes are formed in the body as a result of interlock of antigens with the corresponding antibodies formed against the antigens. In most cases, immune complexes are bound together in a lattice form, blocking the epitopes and the paratopes or the surface molecules regarded as binding sights of the antigens or the antibodies (Shmagel and Chereshnev 2009). It is obvious that the success of serological test lies in its immunological principle which states that antibody binds to its corresponding antigen *in-vivo* or *in-vitro*. This binding should be effective where and when the surface molecules or the binding sights are free (Cynthia *et al.*, 2007). In serum some antigens and antibodies exist in complex form as immune complexes, binding sights are blocked leaving no room for further *in-vitro* attachment of corresponding antigen to antibody, or attachment of antibody to its corresponding antigen (Brunner, 2001). In this study, we decided to precipitate these immune complexes and dissociate the antigen and antibody to produce 'immune solution'. This solution is expected to contain free antigens and antibodies, and can then be used in-lieu of serum in running immunological tests. The importance of this immune solution for immunological techniques was emphasized.

MATERIALS AND METHODS

Sample Collection

10ml of blood was collected from 100 participants from ICT unit of Human Virology Nnamdi Azikiwe University Nnewi Campus. The serum was harvested and stored in duplicates in two sterile tubes, 3mls for HIV screening and 2mls for immune complex precipitation.

Immune Complex Precipitation

The polyethylene glycol (PEG) precipitation technique as described by Bruner (2000), with minor modifications was used. Briefly, 0.5ml of 8% (average molecular weight, 6000) PEG6000; Sigma, St. Louis in 0.1 M borate buffer (pH 8.4) was added drop wise with constant stirring to 0.5ml of serum collected from the participant.

The tubes were vortexed and incubated at 4°C, for 3 hours, and centrifuged at 8,320 *g* for 15 minutes. Supernatants were carefully removed. The resultant pellet was re-suspended and washed twice with 2.0ml of 4% PEG solution in the same buffer, removing the supernatant carefully each time. After the second spin, the solutions were treated with 1ml 0.01M Phosphate Buffer Saline (pH 7.2), and kept as immune solutions in the PBS buffer at 4°C and assayed serologically the following day for HIV, *Plasmodium falciparum* antigens, *Hepatitis B surface antigen* (HbSag), *Hepatitis C virus* (HCV) and *Salmonella typhi* and *paratyphi* antibodies.

Detection of Microbial antigens

The application of Algorithm was observed using membrane based Immunoassay (immunochromatographic) and Abbott determine 1 & 2 for HIV immunoassay**.** The antibodies for other microbial agents (HCV, HBV, *Plasmodium falciparum*) were assayed for in the colorless clear immune solution and unprocessed serum sample, using membrane based immunoassay. Both the immune solution and unprocessed sera samples were used at the dilution of 1:10 The methods were as described by the manufacturer of the Immunoassay kits Global (Acon Incorporated USA) and Abbott determine 1 & 2, using unprocessed serum sample and Immune solution concurrently.

Detection of Salmonella Antigens

Detection of *Salmonella* antigens in the sera samples and immune solutions was done using monoclonal antibody (mabs) (anti-somatic and anti-flagellins igg) (Antec diagnostics United Kingdom), raised against *Salmonella typhi* and *Salmonella paratyphi* antigens. This was reacted against the representatives of known *Salmonella typhi* and *Salmonella paratyphi* antigens (Antec diagnostics United Kingdom) as control; then the known *Salmonella typhi* and *Salmonella paratyphi* antigens were used to screen for *Salmonella typhi* and *Salmonella paratyphi* antibodies in the 100 sera samples and the 100 immune solutions, using tile agglutination method as described by the manufacturer (Antec). Positive sera samples and immune solutions were further subjected to tube agglutination titration method to establish the titre of the antibodies in sera samples and immune solutions in 2 fold dilutions of solutions respectively (1;10, 1:20, 1;40, 1:80, 1:160, 1:320)

RESULTS AND DISCUSSION

Out of the 100 samples analyzed, all the participants that were found positive using unprocessed serum samples, were also positive when immune solution was used. However some participants, that were found negative using unprocessed serum, were found positive using immune solution as follows: 10 (10%) participants were HIV sero-positive for serum sample. On the other hand, 3 additional participants who were previously negative, were HIV positive when immune solution was used, Hence 13(13%) participants were positive under immune solution, for both the global kit and Determine kit. Furthermore, 21(21%) and 26(26%) were positive for malaria parasites in serum and immune solution respectively. 2(2%) and 5(6%) were positive for HCV while 0(0%) and 2(2%) were detected for HBV in serum and immune solution respectively (Table 1).

Detection of Salmonella Species

Disparity in the reaction observed using serum and immune solution was in the level of titre. Out of the 100 sera samples and their 100 alternative immune solutions 36 positive results were obtained at the screening stage. 24(24%) were screened positive for *Salmonella paratyphi* using serum, also 24(24%) were positive when immune solution was used, while 12(12%) and 12(12%) were positive for *Salmonella typhi* in sera and immune solutions respectively. After the titration of the 24 *Salmonella paratyphi* positive sera and immune solutions, 12 sera samples gave 1:80; 8 gave 1:160 and 4 gave 1:320. Using the immune solution, 4 gave 1:80, 4 gave 1:160, 11 gave 1: 320 5 gave 1:640. After the titration of the 12 *Salmonella typhi* positive sera and immune solutions, 4 sera samples gave 1:80, 8 gave 1:160, while in the immune solution, 4 gave 1:160, 6 gave 1:320, 2 gave 1:640 (Tables 2 and 3).

The act of precipitation and quantitative measurement of the immune complexes has been used in detection of immune complexes and in estimation of levels of serum immune complexes in certain disease conditions by many scientists (Onyenekwe *et al.,* 2000, Tanyigna *et al*., 2004). However, little or no attention has been shown in the use of the scientific act in performing routine immunological tests. In this study, this phenomenon was shown to be useful in every immunological tests. Serological tests have shown negative or weakly positive where supposed positive sera samples are applied, resulting to many false negative results (Brunner, 2001). In this work, some results from immune solutions showed positive where corresponding serum samples showed negative. Also results from immune solution showed higher titer where corresponding serum sample showed low titer.

We hypothesized that the free antigens and antibodies in the Immune solution, with exposed surface molecules, enabled effective antigen-antibody binding and more sensitive and specific situation in the immunological techniques. This is opposed to the situation where unprocessed serum with existence of antigen-antibody

complexes is used for serological tests. Suffice it to say that immune complex formation is one of the major limitations encountered in any techniques where principle revolves round antigen antibody reaction. Other limitations may include presence of atypical antibodies that may be competing for binding sights, prozone and

Table 1. The comparison and disparity results of HIV test using serum sample and immune solution concurrently.

Antigens	Serum	Immune solution
HIV	10(10%)	13(13%)
MP	21(21%)	26(26%)
HCV	2(2%)	5(5%)
HBV	0(0%)	2(2%)

Table 2. The comparison of Widal Screening test for *Salmonella species* using sera samples and immune solutions concurrently.

	N	Serum	Immune solution
Agglutination	100	36	36
No agglutination	100	64	64
S. Paratyphi	100	24	24
S. Typhi	100	12	12

Table 3. The comparison of antibody titre for assay of *Salmonella* antibodies in sera samples and immune solutions concurrently.

Titer	1/180	1/160	1/320	1/640
Serum (*S. Paratyphi*)	12	8	4	0
Immune sol. (*S. Paratyphi*)	4	4	11	5
Serum (*S. Typhi*)	4	8	0	0
Immune sol. (*S. Typhi*)	0	4	6	2

post zone phenomena. Other consequences that may ensue due to this development, is poor assessment of epidemiological disease condition in a locality due to false negative and false positive results. Wishfully, we have to progress in this study by trying to use this improved immune solution to detect HIV antigens or antibody at its early stage.

Certainly, the diagnostic limitations experienced during serological tests and in which antigen antibody reaction is expected have led to the development of monoclonal antibody technology to boost specificity and many immunological application exist where this technique has been applied successfully Ezeani *et al.*, 2008. The importance of this exercise is to scientifically establish a route of application in immunology in which specificity and sensitivity of the application is improved. In the same manner, the processing of serum to get immune solution and subsequent use of immune solution in immunological methods would go a long way enhancing immunological techniques both routinely and in research purposes. Studies of equal dilutions (1:10) of serum and ICs made by Brunner and Sigal (2000), revealed greater levels of specific anti-*Borrelia burgdorferi* (anti-Bb) IgM in ICs than in serum, expressed quantitatively by ELISA and qualitatively by immunoblot. Specific antibodies can be sequestered in ics, which make them undetectable in assays using serum samples. Thus, use of IC-derived antibodies may more completely delineate specific humoral immunity and, thereby, more accurately assist in the seroconfirmation of Lime Diseases (LD). Zhong *et al.*, (1997), demonstrated the potential diagnostic advantage of ics and their association with "active disease," in a relatively large study.

Production of immune solution, will certainly free the antigens and antibodies, and expose their epitopes and paratopes respectively. Immune solution is shown to contain the antigen and early formed antibody (especially IgM). Hence enabling antigen and early antibody detection (Brunner, 2001). Earlier, Brunner and Sigal (2000), reported that IC allowed them to "see" more bands in immunoblots than a comparator commercial test, and exhaustive absorption of IC along with a "good" MAb even allowed them to detect *Borrelia burgdorferi* antigen in early infection. While the immune solution is recommended here for high technological methods such as Polymerase Chain Reaction, Western Blotting, Enzyme linked immunosorbent Assay etc, cost effective and less cumbersome methods such as tile and tube agglutination methods, immunochromatographic methods can now be confidently applied in the rural laboratories mainly in developing countries based on the improved specificity and sensitivity obtained using immune solution instead of unprocessed serum.

It is of note that precipitation and separation of antigen-antibody is also important in every serological test and or immunological techniques, as the overall objective of this development is to widen the scope of antigen or antibody detection by way of improving specificity, sensitivity, accuracy and reproducibility of the result.

In conclusion, we conclude that:

1. The immune solution contains free and more sensitive antigens and antibodies than in serum.
2. The immune solution have been found to keep in refrigerator for 6 months and retained its potency.
3. The immune solution contains free particulate antigens, soluble antigens and antibodies. Hence it lends itself to both agglutination and precipitation test.
4. Serum contains only soluble antigens and antibody. Hence can only be used for direct agglutination test only when known particulate antigen is commercially procured. But when only known antibody is available, the serum becomes useless because it does not contain particulate antigen. Hence can only lend itself to precipitation test in this situation. Mean while the immune solution can be used for direct agglutination test on availability of only either known particulate antigen or known antibody.

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