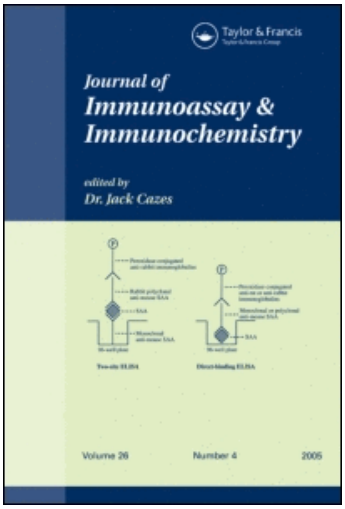

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LASER LIGHT SCATTERING IMMUNOASSAY FOR MALARIA

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ABSTRACT

Laser light scattering immunoassay (LIA) was proposed as a prospective diagnostic method for the detection of antibody (or antigen) by monitoring the agglutination of antigen (or antibody) coated carrier particles using dynamic light scattering (DLS) as probe. LIA is a very sensitive assay as it can detect microscopic immune complexes even when antibody (or antigen) level is low. A sizeable number of human sera collected from malaria endemic areas and hospitals have been analysed by ELISA using Pf parasite lysate or a RESA derived synthetic peptide as antigen parallel to LIA using Pf antigen coated polystyrene latex beads. Comparative analysis of data suggests LIA to be as good as ELISA and possibly better in terms of sen-

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sitivity and simplicity. LIA can be a simple and inexpensive immunoassay suitable for field use and mass application.

KEY WORDS: Laser immunoassay, Malaria, CONTIN

INTRODUCTION

Visual agglutination tests have been used extensively for the detection of antibody and shown to be very useful for the diagnosis of infectious diseases (1-2). Major limitations of the assay are its low sensitivity and specificity. In order to make agglutination visible to the naked eye, a large network of antigen and antibody must form, which requires high antibody concentration and equivalent neutralisation. In a situation of excess antibody or antigen, immune complexes remain small and are not visible to the naked eye. Moreover, high antibody concentration may lead to nonspecific interactions and may compromise the specificity of the assay. Nonetheless, simplicity of agglutination assay makes it a preferred choice over more sensitive and specific assays like enzyme linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) specially in field applications. Both ELISA and RIA involve elaborate experiments and are relatively cumbersome compared to agglutination assays. Past experimental developments in biosensor technology based on laser optics (3-5) have made it possible to detect microscopic immune complexes by laser light scattering based on which LIA are being developed.

LIA as a diagnostic technique was originally proposed by Cohen et al. (4-6) for sensitive detection of antigen-antibody complexes using dynamic light scattering as probe. They showed that this technique was as sensitive as RIA and antibody could be detected at picogram level.

Antibody is a symmetrical molecule that has two antigen binding sites. It can form a bridge between antigen carrier particles, that leads to agglutination. As such a process is invariably associated with a size increment, it can be monitored by light scattering technique which is very sensitive to particle size. We have used LIA for the detection of antibody to *P. falciparum* that causes malaria. The DLS data were analysed using constrained regularization method (CONTIN) (7-9) that gives the relative particle density of various components as function of diameter. The detailed theory and data analysis by CONTIN method in dynamic light scattering (DLS) experiment using malaria antigen was reported earlier (10). Here data on a large number of human sera analysed by ELISA and LIA are presented. We show that LIA can be used as a diagnostic method which is as sensitive as ELISA but much simpler in principle and practice.

MATERIALS and METHODS

The synthetic peptide antigen, Rl (EEBVEGDA-C), *P. falciparum* lysate and the human sera were from Malaria Research Centre, Delhi, India. Polystyrene latex beads were from Sigma Chemical Co. USA. ELISA and agglutination assays were carried out on ELISA Reader 530 from Organon Teknika, Belgium. Agglutination and LIA were done using antigen coated latex beads, which were prepared by incubating the beads overnight with antigen solution in bicarbonate buffer (pH=9.5) at 4^0C. For this, 5 ml of 6 mg/ml antigen solution per ml of 1% aqueous suspension of polystyrene beads were used. Agglutination assays were carried out in transmission mode on the Teknika equipment as per protocol supplied by the manufacturer. Briefly, 100 μl antigen coated beads (1% suspension in 50 mM PBS) was mixed with 25 μl serum in the wells of microtitre plates, and incubated

for 2 hours with occasional shaking. Transmittance at 340 nm at seven points across the well was taken, graphically plotted, average transmission calculated and recorded by the machine. Optimum dilution of serum necessary for agglutination was first established through a checkerboard titration, which included a positive and a negative control sera. Laser light scattering experiments were performed on a home made goniometer unit. One arm of the goniometer was fixed and the He-Ne Laser (Aerotech, USA) operating at 632.8 nm and giving a power output of 15 mW was mounted on it. Another arm of the goniometer housed the photomultiplier tube (PMT) that detected the scattered light as photocurrent. This permitted collection of scattered light at various scattering angles. All our experiments were performed at a fixed scattering angle of 90°. The output from the PMT was passed through a preamplifier-discriminator assembly for signal shaping, amplification and digitization. These signal pulses were fed to a correlator as input. The correlator used was a Brookhaven Instruments, USA Model 9000AT digital Correlator and this recorded intensity autocorrelation of light scattered from the agglutinates. Stringent signal to noise criterion was preset in the correlator to reject data not conforming to a baseline difference between measured and calculated values of $< 1\%$. The correlation data was analysed using the CONTIN software provided by the company. LIA was done at room temperature (25 ± 2^0C) in quartz capillary tubes of sample volume $100\mu l$ and the details of the theory and methods have been described elsewhere (10).

ELISA and LIA were performed on a large number of human sera collected from areas of different endemicity. The study population, finger prick blood sample collection and ELISA method have been reported elsewhere (11). Basically, sera were of four distinct groups.

1. Low Endemic area; Haldwani: Samples were collected at MRC in 1990, when slide positivity rate was low (SPR=12.9) (12).

2. Epidemic area; Raigarh: Samples were collected in 1994 during epidemic.

3. Hindu Rao Hospital, Delhi: Random samples from fever cases with definite parasitimia collected in 1995.

4. Control sample: Blood sample collected from healthy laboratory staff having no history of malaria in last 6-7 years.

All samples on filter paper discs were stored at -20^{0}C until use.

Results and Discussion

Initially, 40 human sera from a known endemic area were checked by ELISA using both Rl and Pf lysate antigens in parallel experiments. Out of these forty samples, 23 were clear positive or clear negative (i.e., cutoff OD $\pm$ 2 SD) in both the assays. These sera were then analysed by agglutination assay. The results of ELISA and agglutination assay is given in Table-1. Four samples from this table (marked S1, S2, S3, S4) were chosen for LIA, which included one clear positive (S2), one clear negative (S4) and two samples (S1, S3) which were clear positive in ELISA but were borderline cases (cutoff $\pm$ 1 SD) in agglutination assay. LIA data showed S1, S2, S3 as sero positive and S4 as sero negative (10) and the results matched with ELISA but not with agglutination assay. These data indicate superior sensitivity of LIA over usual agglutination assay, visual or otherwise. Thus LIA can be used for detection of antibody in the window period, when antibody level is low and immume complexes are of microscopic size, and possibly S1 and S3 were two such samples. One to one correlation with ELISA at same serum dilution suggests sensitivity of LIA is as good as that of ELISA, if not more.

TABLE-I

Sample ID	ELISA Results		Agglutination Results	
	R_1	Pf	R_1	Pf
755 AP	−	−	−	+
468 BP	+	+	+	+
510 CP ($\mathbf{S_1}$)	+	+	+	+/−
344 CP	−	−	−	−
97 AP	−	−	−	−
415 CP	ND	−	+/−	−
741 AP	+	+	+	+
64 CP ($\mathbf{S_2}$)	+	+	+	+
493 CP	+	+	+	+
561 BP	+	+	+	+
267 BP ($\mathbf{S_3}$)	+	+	+	−/+
468 BP	+	+	+	+
402 BP	+	+	+	+
482 CP ($\mathbf{S_4}$)	−	−	−	−
6 PPA	−	−	−	−
262 AP	−	−	−	−
479 BP	−	−	−	−
792 BP (1:4)	−	−	−	−
415 CP	−	−	−/+	+/−
741 AP/4	+	+	+/−	−
97 AP/10	−	−	−	−
260AP/5	−	−	−	−/+
17PP/8	+	+	+	−/+

The above view is further corroborated by the results presented in Tables 2,3 and 4. Table-2 compares ELISA and LIA data on 42 human sera from a low endemic area, Haldwani, where bio-environmental measures for vector control were in operation for several years (11). There was excellent correlation between the two methods. Out of 42 sera, only 6 did not match with either of the ELISA assays. Of these six, three sera (nos. 4, 17 and 25) were slightly positive in ELISA but seronegative by LIA. In

TABLE-II: Haldwani Samples

Sample ID	ELISA		LIA
	Pf	R_1	*Pf*
1	–	–	–
2	–	–	–
3	–	–	–
4	+	+	–
5	++	++	+
6	–	+/–	–
7	–	++	–
8	–	–	–
9	+	+	+
10	–	–	–
11	–	–	+/–
12	–	–	–
13	–	–	–
14	–	–	–
15	–	–	–/+
16	–	–	–
17	+	+	–
18	–	–	–
19	+	+	+/–
20	–	–	–
21	–	–	–
22	–	–	–
23	–	–	–
24	–	–	–
25	+/–	+	–
26	–	–	–
27	–	–	–
28	–	–	–
29	–	–	–
30	–	–	–
31	–	–	–
32	–	–	–
33	–	–	–
34	–	–	–
36	–	+/–	–
37	+/–	+	+/–
38	–	–	–
39	–	–	–
40	–	–	–
42	–	–	+/–
standard +ve	++	++	++

TABLE-III: Raigarh Samples

Sample ID	ELISA		LIA
	Pf	R_1	Pf
1	+	++	++
2	+	++	++
3	+	++	++
4	+	++	++
5	+	++	++
6	+	++	++
7	++	++	++
8	+	++	−
13	+	+	++

ELISA false- positive or false- negative data are not uncommon and therefore these were not serious discrepancies. We have taken false negative as those samples where atleast one of the ELISA tests showed clear negative results, +/− was not considered as false negative. The other three samples (11, 15 and 42) which were borderline in LIA but seronegative in both R1 and Pf - ELISA, were interesting and possibly reflected higher sensitivity of LIA compared to ELISA (5). Table-3 shows analysis of sera from a malaria epidemic area (Raigarh). These were fever cases and all of them were seropositive by both the ELISA assays, whereas by LIA all were highly positive except sample no 8. This could be an example of false- negative in LIA, which might arise out of insufficient time of data acquisition. We observed that due to low laser power (15mW) data acquisition time had to be longer which enables the Correlator to establish a well defined baseline and thus better data accuracy. This problem can be eliminated by using somewhat stronger lasers ($\sim 50mW$).

Table-4, gives analysis of sera from Hindu Rao Hospital of patients with clearly identified parasitemia (slide positivity) and some healthy control

TABLE-IV: Hindu Rao Samples

Sample ID	ELISA		LIA
	Pf	R_1	*Pf*
59*Pf*	–	+/–	+
106 *PV*	+/–	+/–	++
1394 *PV*	+	+	+
1355 *PV*	–	–	++
E5 *PV*	++	++	++
1395 *PV*	+	++	++
1377 *PV*	–	–	++
74 *Pf*	+	–	++
151C	––	––	––
86 *Pf*	+	+	+
1398 *PV*	+	++	++
1386 *PV*	++	+	++
93 *Pf*	++	++	++
81 *Pf*	++	+	++
1357 *PV*	++	++	++
1356 *PV*	++	++	++
1381 *PV*	++	+	++
91 *Pf*	++	++	++
50 *Pf*	–	+/–	+
64 *Pf*	+	+	++
101 C	–	–	–
23 *Pf*	+/–	–	+
76 *Pf*	+/–	+	+
180 C	+/–	+	–
57 *Pf*	+	+	+
860 *PV*	+/–	+/–	+
49 *Pf*	+	+	++
96 C	–	+/–	–
80 *Pf*	++	+/–	+
1394 *PV*	+/–	+	+

(marked C) by the three assays. There was 100% correlation between slide positivity and LIA, whereas ELISA data gave some false- positive (sample nos. 180C, 96C) and some false- negative (59Pf, 1377PV, 1355PV, 74Pf, 50Pf and 23 Pf) results in our hands. These data clearly demonstrate the edge LIA has over ELISA in sensitivity, which corroborates previously published observations (4-5).

We have addressed here only the sensitivity aspect, but not the specificity issue, albeit we realised that both need to be examined for establishing a successful diagnostic methodology. Our contention was that specificity of LIA will be determined solely by the specificity of the antigen (or antibody) used. We find better correlation of LIA with Rl-ELISA. Rl was a much better defined antigen than Pf lysate, and was more specific (13) and data here support our contention. Nevertheless, we will be addressing the specificity issue in a subsequent study. Here we established that LIA was a very sensitive but simple assay, which can be used for any infectious disease situation including HIV. LIA can be used both for antibody or antigen detection on mass scale. This advantage is specially relevant for AIDS screening in blood banks and hospitals where ideally the blood should be analysed both for p24 antigen during the initial "window period" as well as for the detection of conventional antibodies simultaneously with none-too-expensive tests on a large scale and LIA is well suited for that. On the contrary, ELISA is a cumbersome procedure involving costly enzymes and enzyme antibody conjugates, and requires several steps to come to final results. Agglutination on the other hand is much simpler where blood serum and antigen are mixed which causes the antibody to agglutinate. On this principle LIA happens to be far more simpler procedurally compared to RIA or ELISA.

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REFERENCES

1. Atlas, R.M., Microbiology: Fundamentals and applications, Macmillan Publishing Company, New York, 1989, pp. 582-9.

2. Kemp, B.E., Rylatt, D.B., Bundesen, P.G. et al. Autologus red cell agglutination assay for HIV-1 antibodies : simplified test with whole blood. Science 1988;241: 1352-4.

3. Turner, A.P.F, Karube, I. and Wilson. G.S. Biosensors: Fundamentals and applications, Oxford Science Publication, New York, 1987, pp. 655-701.

4. Cohen. R.J. and Benedek.G.B. Immunoassay by light scattering spectroscopy. Immunochemistry 1975;12:963-6.

5. Von Schulthess. G.K., Cohen. R.J, Sakato. N. and Benedek. G.B. Laser light scattering spectroscopic immunoassay for mouse IgA. Immunochemistry 1976;13:955-62.

6. Von Schulthess. G.K., Cohen. R.J. and Benedek. G.B. Laser light scattering spectroscopic immunoassay in the agglutination-inhibition mode for human chorionic gonadotropin (hCG) and human luteinizing hormone (hLH). Immunochemistry 1976;13:963-6.

7. Provencher S.W. Contin: a general purpose constrained regulariza-

tion program for inverting noisy linear algebraic and integral equations. Comput.Phys.Commun. 1982;27:229-42.

8. Provencher S.W. A constrained regularization method for inverting data represented by linear algebraic or integral equations. Comput. Phys. Commun.1982;27:213-27.

9. Provencher S.W. Users Manaual for CONTIN, Technical Report EMBL-DA05, Queens University Belfast, Belfast, 1982.

10. Antony, T., Saxena, A., Roy, K.B. and Bohidar, H.B. Laser light scattering immunoassay: an improved data analysis by CONTIN method. J. Biochem. Biophys. Methods 1998;36:75-85.

11. Roy, A., Sharma, V.P. and Chauhan, V.S. The use of peptide ELISA in determining malaria endemicity. J. Immunol. Methods 1994;167:139-43.

12. Roy, A., Biswas, S., Shukla, R.P. and Malhotra, M.S. Assessment of malaria Transmission through seroepidemiology of children population. J. Parasitic Diseases 1996;20:53-6.

13. Roy, A., Biswas, S., Kabilan, L. and Sharma, V.P. Application of simple peptide ELISA for stratification of malaria endemicity. Indian J. Malariology 1995;32: 164-73.