

## A Comparative Study of Semi Quantitative Latex Agglutination Test and Quantitative Turbidimetric Immunoassay Method for the Detection of C-Reactive Protein from Human Sera.

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### Abstract :

**Introduction :** C-reactive protein (CRP) is a major prototypical acute-phase protein (APP) in humans. Its short half-life makes it a particularly good marker to detect and follow any disease activity. **Aim :** The aim of this study was to compare semi quantitative slide Latex Agglutination test (LAT) with quantitative Turbidimetric Immunoassay (TIA) method for the detection of CRP. **Materials and Method :** The sera of 400 patients clinically suspected to have systemic inflammation were tested using two methods. **Results :** From a total of 400 patients, 158 (39.50%) were positive by slide LAT & 276 (69.00%) were positive by TIA. Slide LAT gave false negative results for 118 patients. The sensitivity of the slide LAT was only 57.24% in comparison to the TIA (100%). Both the tests were equally specific (100%). **Conclusion :** The study revealed a significant difference between two methods.

**Key Words :** CRP, slide Latex Agglutination test, Turbidimetric Immunoassay

### Introduction :

With the scientific advancement effective antibiotics are available for treatment but early diagnosis represents a major challenge due to non-specific nature of signs and symptoms.<sup>(1,2,3)</sup> C - reactive protein (CRP) is the most extensively studied acute-phase reactant as its blood concentration increases from less than 1 µg/mL to as high as 600–1000 µg/mL during the height of an acute-phase response. The half-life of CRP in the circulation is about 19 hours. Due to wide availability of simple, fast & cost-effective laboratory methods, CRP detection is the preferred test in evaluation of inflammatory conditions.<sup>(4,5,6)</sup> Apart from indicating inflammatory disorders, CRP measurement helps in differential diagnosis, in the management of neonatal septicemia and meningitis where standard microbiological investigations are time consuming. Its use in postoperative surveillance is also of great importance.<sup>(7,8)</sup>

Until the late 1970s, CRP was measured using qualitative or semi-quantitative laboratory technique, most commonly latex agglutination, which precluded its use as differential diagnostic test because any degree of inflammation produced positive results.<sup>(9)</sup> Presently, accurate and rapid quantitative measures of CRP are obtained using laser nephelometry, turbidimetric immunoassay, and enzyme

immunoassay. The quantitative method is widely used in developed countries because it provides rapid, highly sensitive and specific results.<sup>(10,11)</sup> We have therefore investigated the use of a quantitative Turbidimetric Immunoassay (TIA) & compared it with slide Latex Agglutination test (LAT).

### Materials and Methods :

Total 400 serum samples were collected from the patients, clinically suspected to have systemic inflammation at tertiary care hospital of Ahmedabad city of Gujarat, India. The study was carried out from January 2013 to May 2013. CRP concentrations were determined in serum samples using commercial slide LAT (RHELAX-CRP, Tulip) and TIA (Quantia-CRP UV, Tulip). Testing was done according to the manufacturer's guidelines for both the tests. TIA was performed by automated ERBA XL-640 machine. All the statistical data (sensitivity, specificity, student T-test, CV, correlation coefficient) were calculated by using the SPSS 15.0 & MedCalc software.

### Results & Discussion :

The Turbidimetric immunoassay method is more powerful than the semi-quantitative methods and is used in human medicine of share their many qualities such as the precision, the speed, the quantification and the possibility of being automated.<sup>(12, 13)</sup> The excellent reproducibility of results read by an instrument and the expression of the results in quantitative international units are an advantage over traditional agglutination techniques, where the subjective interpretation of slide LAT causes problems with accuracy and precision.<sup>(14)</sup>

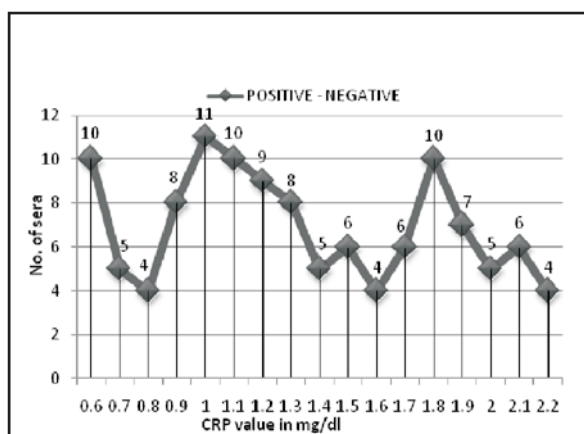
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In this study, from a total of 400 patients, 216 were males & 184 were females. Majority of the patients (65.25%) were between the age group of 0 to 15 years. About 150 (38%) patients were from the ward, 25 (6%) from the ICCU, and 225 (56%) from the OPD.

**Graph 1 : CRP values of the sera which were slide LAT negative & TIA positive**



**Table 1: Results of TIA versus slide LAT.**

Test with Interpretation		Slide Latex agglutination test		
		Positive	Negative	Total
Turbidimetric immuno assay	Positive	158	118	276 (69.00%)
	Negative	0	124	124 (31.00%)
	Total	158 (39.50%)	242 (60.50%)	400 (100.00%)

Out of 400 sera, 276 (69%) were positive & 124 (31%) were negative by TIA while 158 (39.5%) were positive & 242 (60.5%) were negative by slide LAT (Table 1). Slide LAT gave false negative results with 118 sera. Out of these 118 sera which were negative with slide LAT, 38 sera were having borderline CRP value of (0.6-1.0mg/dl), 38 were within the range of (1.1-1.5mg/dl), 32 were within the range of (1.6-2.0mg/dl), and 15 were between (2.0-2.2mg/dl) by TIA method. (Graph 1)

Although kits for the slide LAT are easily available & slide LAT is cheap due to no need of equipment for it, there are many merits of TIA over slide LAT. TIA was performed with automated ERBA XL-460 machine, in which quantification can be performed automatically for the CRP values up to 10mg/dl from the sera without dilution in this study. TIA is also more sensitive than visual detection of the aggregates. A spectrophotometer can also be used instead of this costly automated machine for TIA. The major limitations with slide LAT are that quantitation was only possible after serial dilution of the serum & subjective variation in looking for the titre showing absence of agglutination.

With slide LAT only 5 or 6 sera could be tested on latex agglutination card at a time while with the TIA more than 50 sera could be tested at a time using the automated machine in this study. Only one technical staff can operate this automated machine as this machine not only measure transmitted light automatically at a desired time but also dilute, pipette, and transfer to the cuvette the convenient volumes of reagents buffers and samples, incubate at a programmed temperature and make the necessary calculations using the selected algorithms and calibration curves.

In this study, sensitivity of the slide LAT was only 57.24% in comparison to the TIA (100%) & specificity of both the tests was equal. (100%).

The Correlation coefficient  $r = 0.928$  for the test of correlation indicates that the optical densities all obtained are in the field of correlation. So it showed that both the methods were highly correlated.

**Table 2: Comparison of the two methods. (Unpaired t Test)**

Methods	Average	T Value	P
Turbidimetric Immunoassay	5.97	9.241	0.000
Latex Agglutination	3.07		

The averages obtained by the two methods were statistically different with  $P < 0.05$  and T calculated value (9.241) was more than T tabulated value (3.29). So null hypothesis was rejected & our study revealed that TIA is highly significant method than slide LAT ( $T_c > T_t$ ) (Table 2).

TIA method gave  $CV=2.12\%$  comparable with those provided by the manufacturer  $CV=2.08\%$  as well as comparable with slide LAT,  $CV=2.47\%$ . So it showed that TIA was more consistent and more homogeneous method in this study.

A higher rate of positivity was observed in TIA in our study (69.5%); A finding supported by AÏKOU Nicolas et al. who reported 81% positivity in TIA. <sup>(15)</sup> Kari Pulkki et al. <sup>(16)</sup> observed that slide LAT shown 80 discordant results out of 708; in our study we also found 118 discordant results out of total 400 sera with slide LAT.

Easy adaptation of TIA method to the clinical laboratory instrumentation is also advantageous as we performed TIA method with the help of automated ERBA XL-460 machine & as observed by F.-Javier Gella et al. <sup>(17)</sup> Pranav R. Naik et al. <sup>(18)</sup> noted that both methods are highly correlated as in this study.

Our results are in concordance with findings of study carried out by other authors. AïKOU Nicolas et al.<sup>(15)</sup> concluded that turbidimetry is more powerful than the method of agglutination. Kari Pulkki et al.<sup>(16)</sup> concluded that CRP latex slide test is not useful in emergency laboratory in hospital material with a high incidence of bacterial infections & J.L. Ortega-Vinuesa et al.<sup>(19)</sup> also concluded that turbidimetry allows researchers to perform numerous reliable immunoassays in a very short time.

### Conclusion :

Based on the comparative analysis in this study, we conclude that slide Latex agglutination test & Turbidimetric immunoassay method are equally specific but Turbidimetric immunoassay is more sensitive. Turbidimetric immunoassay is also easier to perform & allows processing of hundreds of samples in a short time, making it suitable for laboratories with large diagnostic workloads. The advantage of automation with Turbidimetric immunoassay also saves the investment in new instrument & personnel.

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