

## “COMPARISON OF A RAPID SEMI-QUANTITATIVE LATEX AGGLUTINATION SLIDE METHOD AGAINST QUANTITATIVE PARTICLE ENHANCED TURBIDIMETRIC IMMUNOASSAY FOR MEASUREMENT OF C-REACTIVE PROTEIN”

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### Abstract:

Serum C - reactive protein (CRP) is an acute phase marker in humans that is useful for the diagnosis and monitoring of inflammatory disease. CRP measurement also helps in differential diagnosis, in the management of neonatal septicaemia and meningitis where standard microbiological investigations are very much time-consuming. Reliable method (Automated particle enhanced turbidimetric immunoassay) are preferable for routine evaluation of human serum CRP levels. Using such expensive methods in health centres in rural areas is not possible. The aim of this to evaluate whether human CRP levels could be measured by rapid method (Latex agglutination slide method) and compared with reliable method (particle enhanced turbidimetric immunoassay). The study included 400 participants, who attended Shree Krishna Hospital, Karamsad, whose CRP levels were done by particle enhanced turbidimetric immunoassay method in Siemens Dimension Clinical Chemistry Analyzer, RxL and Xpand and compared by rapid method (Latex Agglutination slide method), that will be useful in health centres in rural areas where expensive instruments are not available. We used SPSS for data analysis and Endnote X7 for references management. In present study, out of 400 (217 male and 183 female) participants, we found 337 participants had positive CRP levels ( $\geq 0.3$  mg/dl) and 63 participants had negative CRP levels ( $< 0.3$  mg/dl) by particle enhanced turbidimetric immunoassay method, whereas 303 had positive CRP levels ( $\geq 0.6$ mg/dl) and 97 had negative CRP levels ( $< 0.6$ mg/dl) by Latex Agglutination slide method. The correlation was positive between two methods with ( $r = 0.764$ ,  $P$ -value  $< 0.0001$ ). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 89.9%, 100%, 100%, and 64.94%, respectively, for the Latex Agglutination slide method, and 100%, 64.9%, 89.91% and 100%, respectively, for the particle enhanced turbidimetric immunoassay method. Serum C- reactive protein (CRP) levels can be reliably measured using the particle enhanced turbidimetric immunoassay method, but it is expensive, so we can use Latex agglutination slide method in rural areas for primary diagnosis only.

**Key Words:** CRP, Latex Agglutination slide method, particle enhanced turbidimetric immunoassay method.

### Introduction

The C - reactive protein test measures the level of C - reactive protein (CRP) in the blood. C-reactive protein (CRP) is one of the acute-phase proteins synthesized by the liver(1). Its production is controlled primarily by interleukin-6. It's sent into the bloodstream in

response to inflammation. C-reactive protein (CRP) is one of the acute phase proteins and is a reliable indicator of disease activity in various clinical cases. Chronic inflammatory disorders, including autoimmune diseases and malignancy, can produce persistent increases of serum CRP concentrations. Traditionally, CRP has been used clinically for

diagnosis and monitoring of autoimmune and infectious disorders including infections caused by bacteria, fungi, and viruses(2). Routine automated methods for CRP quantification in the clinical laboratory typically have limits of quantification of 3–8 mg/L(3, 4).

Among the methods used to measure CRP in serum are radioimmunoassay (15), radial immunodiffusion (5), latex agglutination (6), nephelometry (5), particle-enhanced turbidimetry (7)and enzyme immunoassays (8).

Different methods for quantifying CRP in serum have been used. Studies conducted with apparently healthy individuals require high-sensitivity CRP (hs-CRP) methods. These high-sensitivity methods initially used ELISA methodology, and a single in-house ELISA assay was used for several population studies.

This methodology is primarily intended for research and is not ideal for routine use in highly automated clinical laboratories. Traditional CRP methods in the clinical laboratory lack the desired sensitivity and, therefore, are unsuitable for the purpose of predicting future risk of coronary events in apparently healthy individuals. A latex-enhanced immunonephelometric hs-CRP method has recently been evaluated and validated clinically(9, 10)

Recently, several automated immunoturbidimetric and immunoluminometric hs-CRP assays have been developed and are commercially available. These assays have improved sensitivity and precision at low concentrations of CRP.

In this study, we will evaluate the serum CRP levels by rapid method (Latex Agglutination method) and compare with reliable method (particle enhanced turbidimetric immunoassay (PETIA)).

**MATERIALS AND METHODS:**

The study included 400 participants, who attended Shree Krishna Hospital, Karamsad, whose measurement of CRP levels were done by particle enhanced turbidimetric immunoassay (PETIA) method in Siemens Dimension Clinical Chemistry Analyzer, RxL and Xpand. CRP concentrations were determined in serum samples using Latex Agglutination slide method (RHELAX-CRP, Tulip) and RCRP kit from Siemens Company.

The study was carried out from June 2017 to April 2019 in the department of biochemistry at PSMC, Karamsad.

Testing was done according to the manufacturer's guidelines for both the tests kits. The CRP was performed by automated Siemens Dimension RxL and Xpand machines. Quantitative RCRP test is working on the principle of particle enhanced turbidimetric immunoassay technique. Synthetic particles coated with antibody to C - reactive protein aggregate in the presence of C - reactive protein in the sample. Increase in turbidity is directly proportional to concentration of CRP in the sample. If the CRP concentration is greater than or equal to 0.3 mg/dl, the test is positive, whereas if the CRP concentration is less than 0.3 mg/dl, the test is negative(11).

CRP values were estimated by semi quantitative Latex Agglutination slide method.

RHELAX CRP slide test used for the detection of CRP is based on the principle of Agglutination. The test specimen (serum) is mixed with RHELAX CRP Latex reagent and allowed to react. If the CRP concentration is greater than 0.6 mg/dl, a visible agglutination is observed. If the CRP concentration is less than 0.6 mg/dl, no agglutination is observed(12).

**Calculate the sensitivity and specificity:**

**Table No. 1. Existing diagnosis criteria/ gold standard**

		Existing diagnosis criteria/ gold standard	
		Positive	Negative
Evaluated test	Positive	a (True positive)	b (False positive)
	Negative	c (False negative)	d (True negative)

Following performance parameters were done by calculated for the evaluated test:

- Sensitivity:  $a/a + c \times 100$
- Specificity:  $d/b + d \times 100$
- Positive Predictive Value (PPV):  $a/a + b \times 100$
- Negative Predictive Value (NPV):  $d/c + d \times 100$

We compared the result we found from different methods for CRP measurement. The study was approved by the institutional ethics committee at PSMC, Karamsad. All the statistical data (sensitivity, specificity, student T-test and correlation coefficient) were calculated by using the SPSS software.

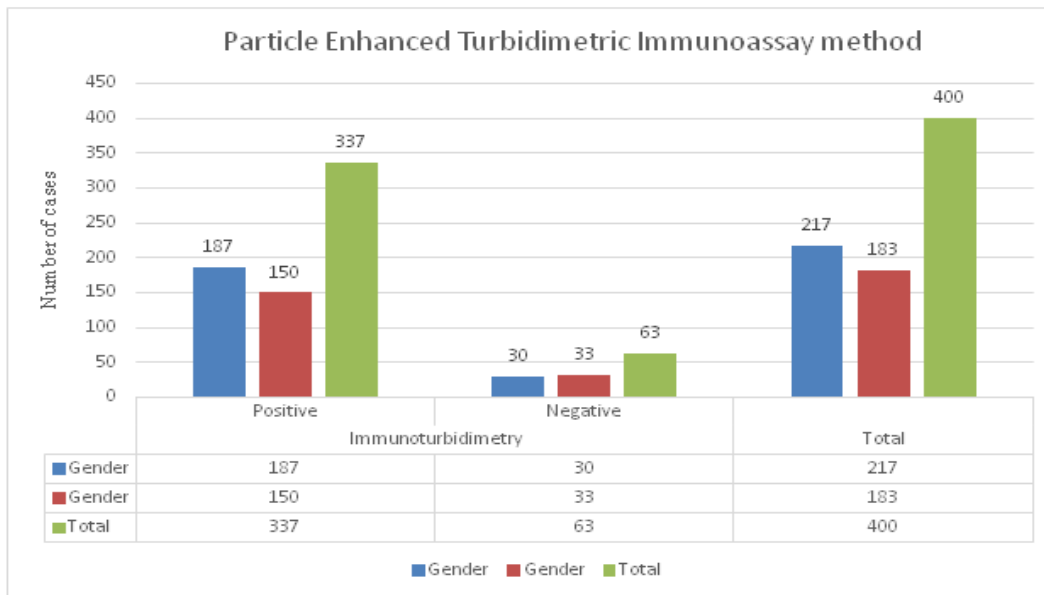
**RESULTS**

In this study, we collected four hundred human serum samples from Shri Krishna Hospital, Karamsad, of the 400 participants, 217 were males and 183 were females.

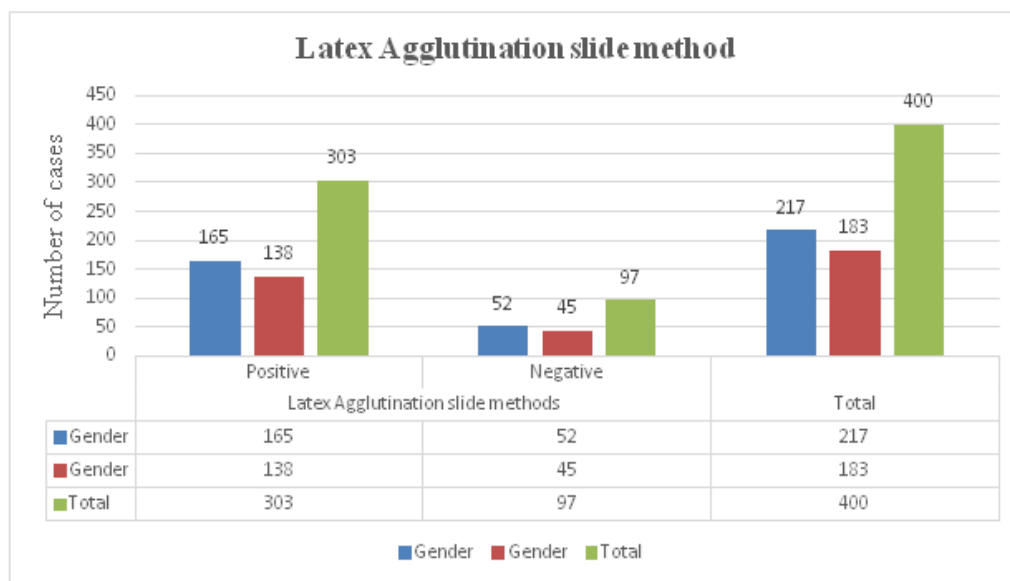
The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 89.9%, 100%, 100%, and 64.94%, respectively, for

the Latex Agglutination slide method, and 100%, 64.9%, 89.91% and 100%, respectively, for the particle enhanced turbidimetric immunoassay method.

The correlation of particle enhanced turbidimetric immunoassay method with Latex Agglutination slide method for CRP measurement is positively significant, Pearson Correlation ( $r$ ) = 0.764 with P-Value <0.0001.



**Figure 1: Distribution of the results of the particle enhanced turbidimetric immunoassay for CRP measurement according to gender.**



**Figure 2: Distribution of the results of the Latex Agglutination slide method for CRP measurement according to gender.**

**Table No: 1 Correlation between particle enhanced turbidimetric immunoassay with Latex Agglutination slide method**

	Latex Agglutination slide method	particle enhanced turbidimetric immunoassay
Pearson Correlation	1	0.764**
Sig. (2-tailed)		<0.0001
N	400	400
Pearson Correlation	0.764**	1
Sig. (2-tailed)	<0.0001	
N	400	400
**. Correlation is significant at the 0.01 level (2-tailed).		

**Table No: 2 Sensitivity and specificity for particle enhanced turbidimetric immunoassay with Latex Agglutination slide method**

Evaluated test	Latex Agglutination slide methods			Total
		Positive	Negative	
Particle Enhanced Turbidimetric Immunoassay	Positive	303	34	337
	Negative	0	63	63
Total		303	97	400

From above table Sensitivity 100%, Specificity 64.9%, PPV 89.91% and NPV 100%

**Table No: 3 Sensitivity and specificity for Latex Agglutination slide with particle enhanced turbidimetric immunoassay methods**

Evaluated test	Particle Enhanced Turbidimetric Immunoassay			Total
		Positive	Negative	
Latex Agglutination slide methods	Positive	303	0	303
	Negative	34	63	97
Total		337	63	400

From above table Sensitivity 89.9%, Specificity 100%, PPV 100% and NPV 64.94%

**Table No: 4 Distribution of the results of the Latex Agglutination slide method and Particle Enhanced Turbidimetric Immunoassay for CRP measurement according to gender**

Particle Enhanced Turbidimetric Immunoassay and Latex Agglutination slide method						
		Particle Enhanced Turbidimetric Immunoassay		Latex Agglutination slide methods		Total
		Positive ≥ 0.3 mg/dl	Negative < 0.3 mg/dl	Positive ≥ 0.6 mg/dl	Negative < 0.6 mg/dl	
Gender	Male	187	30	165	52	217
	Female	150	33	138	45	183
Total		337	63	303	97	400

In this table, we found 337 participants had positive CRP levels (≥0.3 mg/dl) and 63 participants had negative CRP levels (<0.3 mg/dl) by Particle Enhanced Turbidimetric Immunoassay whereas 303 had positive CRP levels (≥0.6mg/dl) and 97 had negative CRP levels (< 0.6mg/dl) by Latex Agglutination slide method.

## DISCUSSION

Many researchers have shown that serum CRP concentration is a useful acute phase marker that is useful for routine diagnosis.

In the routine clinical laboratory, serum CRP is usually measured using turbidimetric or nephelometric immunoassays(5). However, these techniques are often not affordable in low-income countries. In the district hospitals of these countries, because of limited resources health care systems patients suffering from fever are often treated empirically with antibiotics based on clinical suspicion only, without reference to laboratory evidence.

Also several previous studies that examined serum CRP concentrations, using highly sensitive ELISA, nephelometric, and turbidimetric methods<sup>(13)</sup>, but that is expensive and unsuitable for rural areas.

Enhanced immunoturbidimetry assay was utilized as a reference method because it was widely adopted as a gold standard test for in vitro diagnostic(14). However, the semi-quantitative slide method is rapid and inexpensive method to measure serum CRP concentration.

The results showed that the latex agglutination slide test was not a reliable method to test serum CRP levels when compared with immunoturbidimetry, but the degree of agglutination gives a crude indication of the CRP level for the initial diagnosis. Latex agglutination is frequently used as a rapid and simple screening test for elevated levels of CRP. However, false negative results may occur in this method and are reported as false negative results. Therefore, the manufacturers recommend re-testing any negative serum after dilution to avoid false negative results. The latex agglutination test has the advantage of being semi-quantitative if the serum is tested(15). This is what our study supports, we found that 34 out of 400 cases had positive results using particle enhanced turbidimetric immunoassay, but those were negative with the latex agglutination slide test.

That is similarity with previous study done by Manisha N, et.al, they found 158 (39.50%) were positive out of 400 patients, by latex agglutination Slide method & 276 (69.00%) were positive by TIA. The latex agglutination Slide method gave false negative results for 118 patients(16).

We found a test with 89.9% sensitivity correctly identifies all patients with the positive results for

Latex Agglutination slide method (true positives), but 10.1% with the results go undetected (false negatives). A high sensitivity is clearly important.

P.R. Naik, et.al. (2013), concluded that the correlation between immunoturbidimetric and latex agglutination method in both normal abnormal samples was very high(17). This agreement with our study, the correlation between particle enhanced turbidimetric immunoassay and Latex Agglutination slide method was positively significant, Pearson Correlation (r) = 0.764 with P-Value <0.0001.

## CONCLUSION

In conclusion, the particle enhanced turbidimetric immunoassay we evaluated exhibited some differences in results for CRP measurement when compared with Latex agglutination slide method. The serum CRP levels can be reliably measured using the particle enhanced turbidimetric immunoassay, but it is costly, so we can use Latex agglutination slide method in rural areas for initial diagnosis only.

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