

A DISSERTATION ON

**Comparative Analysis of Semi-Quantitative CRP Test using Latex Agglutination Method and  
Quantitative CRP using Neflometric Method**

SUBMITTED TO THE

DEPARTMENT OF BIOSCIENCES

INTEGRAL UNIVERSITY, LUCKNOW



IN PARTIAL FULFILMENT

FOR THE

DEGREE OF MASTER OF SCIENCE

IN

BIOTECHNOLOGY

BY

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UNDER THE SUPERVISION OF

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## **DECLARATION**

I, Ishika Agrawal declare that this submission is my own work under the supervision of Prof. Jyotsana Agarwal (Professor and HOD), Department of Microbiology, Dr. RMLIMS, Lucknow. I have taken four month of training at the Microbiology Lab with the supervisor at the center.

I further declare that to the best of my knowledge the project report is original and it does not contain any part of any work and has not been submitted in part or full to any other institute or university for award or any kind of degree without proper citation.

Date :

Place : Lucknow

**Ishika Agrawal**



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### TO WHOM IT MAY CONCERN

This is to certify that **Ms. Ishika Agrawal** student of M.Sc. Biotechnology (IV Semester), Integral University has completed her four months dissertation work entitled “**Comparative Analysis of Semi-Quantitative CRP Test using Latex Agglutination Method and Quantitative CRP using Neflometric Method**” successfully. She has completed this work from 01<sup>st</sup> Feb-31<sup>st</sup> May 2022 under the guidance of **Prof. Jyotsana Agarwal**. The dissertation was a compulsory part of her M.Sc. degree.

I wish her good luck and bright future.

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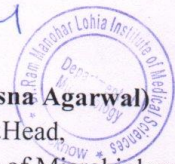
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Date: 31/05/2022

**TO WHOM IT MAY CONCERN**

This is to certify that **Ms Ishika Agrawal** of M.Sc Biotechnology in Integral University Lucknow, has undergone a short term training in the Department of Microbiology, Dr RMLIMS, Lucknow and has worked on the topic "*Comparative Analysis of Semi Quantitative Method and Quantitative CRP using Neflometric method.*" for a period from 01/02/2022 to 31/05/2022.

  
  
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**CERTIFICATE**

This is to certify that the present work entitled “ **Comparative Analysis of Semi-Quantitative CRP Test using Latex Agglutination Method and Quantitative CRP using Neflometric Method** ” has been carried out by Ishika Agrawal, herself under our direct guidance and supervision.

It is further certified that candidate has also fulfilled all the pre requisites necessary for the submission of this project.

**CHIEF SUPERVISOR**

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## **ACKNOWLEDGEMENT**

This project is highly indebted to its existence to the help, support and inspiration of many people. The project work calls for study of particular aspects and one needs support from number of many persons whose contributions in assorted ways the work and the making of the synopsis deserved special mention.

In the first place I would like to thank two people that aided me with my report. I would like to thank **Dr. Snobir Mir, HOD, Department of Biosciences**, Integral University, Lucknow and **Dr. Jyotsana Agarwal, HOD, Department of Microbiology**, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow who give assistance to his best at every step.

I would like to express my sincere thanks and deep sense of gratitude to **Dr. Akansha Gupta, (Assistant Professor, Department of Microbiology)** and **Dr. Jyotsana Agarwal, (Professor and Head, Department of Microbiology)** my guides for their unlimited and unbelievable support and encouragement. I give special thanks for their valuable advices in completion of my project work.

I also would like to thank **Dr. Vineeta Mittal, Dr. Jaya Garg, Dr. Manodeep Sen** and **Dr. Anupam Das** for their valuable support throughout my work.

I would like to thank all my friends for their encouragement and help during my project work. Last but not least I would like to thank my parents for their blessings and help in my project work.

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## **1.KEYWORDS**

- C – Reactive Protein (CRP)
- Inflammation
- Agglutination
- Coagulation
- Latex Method
- Neflometric Method



# **INTRODUCTION**

## **2.INTRODUCTION**

C-Reactive Protein (CRP) was uncovered by Tillett and Francis in 1930. The name CRP was originated because it was first analysed as a substance in the serum of patients with acute inflammation that reacted with the “C” carbohydrate antibody of the capsule of Pneumococcus.

CRP is a pentameric protein synthesized by the liver, whose level increases in response to inflammation. It is an acute phase reactant protein that is chiefly produced by the IL-6 action on the gene responsible for the transcription of CRP during the acute phase of an infectious process.

As high levels of CRP in blood are solid indicators of inflammation, a CRP test can be important initial step in analyzing medical conditions that cause inflammation. This includes infections and autoimmune diseases, such as lupus.

Although the CRP test cannot disclose where the inflammation is occurring or what is causing it, results can make your doctor to think into the direction of possible doubt.

CRP has both pro-inflammatory and anti-inflammatory estates. It plays a role in the identification and clearance of foreign pathogens and damaged cells by hitching to phosphocholine, phospholipids, histones, chromatids and fibronectin.

CRP can trigger the classic complement pathway and also active phagocytic cells via Fc receptors to encourage the removal of cellular trash and spoiled or apoptotic cells and foreign pathogen. It can become diseased, however, when it is triggered by auto-antibodies showing the phosphocholine arm in auto-immune processes, such as idiopathic thrombocytopenic purpura (ITP).

CRP can also deteriorate tissue damage in certain cases by stimulation of the complement system and thus inflammatory cytokines. As correlated to the erythrocyte sedimentation rate, an indirect test for infection, the levels of CRP increases and decreases very frequently with the start and elimination of the damage stimulates respectively. Constantly boosted CRP levels can be seen in chronic inflammatory conditions such as chronic infections or inflammatory conditions such as chronic infections or inflammatory arthritis such as Rheumatoid Arthritis.

CRP that has been sustained throughout the development is a host defense molecule. Its attraction towards phosphocholine-ligands, such as modified low-density lipoprotein, and apoptotic cells leads to the disguising of these substances that have the potentials to otherwise enthal in pernicious activities. Complement stimulation by CRP complexes and the conversion by CRP of complement activation by its ligands add up to its favourable effects.

There are various causes of an boosted C-reactive protein, these include acute and chronic conditions, and these can be infectious or non-infectious. However, noticeably boosted levels of CRP are most constantly linked with an infectious cause such as pathogen associated molecular pattern recognition.

In the presence of CRP, production of membrane-damaging last product of the complement pathway is arrested. CRP is now-a-days presenting as an marker of cardiovascular diseases, but to determine the role of CRP in antherosclerosis, a drug that can lower cholesterol levels, but not the CRP levels, is needed for analyzation.

CRP, an inflammatory indicator, may also play proinflammatory role in triggering monocyte chemostatic protein. Anti-antherosclerosis drugs may be imposing some of their advantageous effects by holding on the hazardous effects of CRP.

### **CRP TEST :-**

The CRP test is a general indicator for infection. It is used to conclude if someone's symptoms are related to an infectious or non-infectious condition. The results, along with other findings, can help restricted the possible effects.

Although there are some kinds of limitations for the test through which it can disclose the trustworthy way to measure infection. The higher the CRP levels, the more amount of infection in the body.

This test is executed when the doctor doubts acute or chronic infection. This test computes the amount of C-reactive protein in the human blood. In response of inflammation CRP is transfer into bloodstream.

A CRP-test may be used to check conditions that cause infection. Doctors need to perform this test if they found some conditions related to serious bacterial infections.

The CRP test can help to identify a wide array of medical conditions, including –

- 1). Asthma
- 2). Rheumatoid Arthritis
- 3). Bronchitis
- 4). Cancer
- 5). Heart Attack
- 6). Pneumonia

7). Diabetes

8). Pancreatitis, and many more.

As someone have already been analyzed with an infection or chronic disease, this test may be used to observe the treatment. CRP levels increase and decrease depending on how much infection you have, if levels go down, it's a sign that your treatment for inflammation is working. The CRP level can also tell if the inflammation is acute (severe and sudden, such as allergic reaction) or chronic (persistent, such as diabetes).

### **CRP Quantitative Test :-**

The CRP quantitative test measures the levels of CRP in the blood. Quantitative CRP determines the presence of infectious response in the blood. Increase in quantitative CRP indicates a severe inflammatory reaction in the body. Decreasing levels of CRP shows recovery from the infections.

So, the CRP quantitative tests analyze treatment and progress the ongoing disease conditions. The quantitative test of CRP measure the exact amount of CRP present in the blood of the respective patient.

CRP quantitative test denotes its positive result above the 6mg/L. The result shown on screen analyzes as positive result but the result very near 6mg/L is unable to be seen onto the latex procedure of CRP. Thus, it is very compulsory to analyze the quantitative CRP test of surgery patients to monitor their progress before and after surgery and also during the medications of various patients.

Quantitative CRP test is done using the nephelometric method which in process uses a semi-auto analyzer machine to analyze the results of the patients that contains a sipper pipe to sip the sample and distilled water inside the machine to analyze the serum sample as if the result is positive or negative.

The results of CRP quantitative has to be analyzed by using graph technique that uses to drop up or down to analyze as if the results are positive or negative but sometimes we uses the graph to analyze the standard of the kit that we uses in the test to check whether the kit will be able to provide the correct result or not. As the kit used in this test have to be stored at +2 to +4 degree celcius and can also be used till 6 months after the opening of the kit.

CRP quantitative test has to be done perfectly by washing the machine perfectly by sipping the distilled water into the machine and maintain it properly to get the appropriate results of the test by using the blood serum in a proper amount.

### **CRP Semi-Quantitative Test :-**

CRP semi-quantitative test are also known as Dilution Test of CRP by using normal saline solution from the laboratory and mix the samples with the saline to get the result of the test.

CRP semi-quantitative has to be done only by those samples that tests positive either in the latex or in the quantitative test. So, by using this test one can conform the positivity of the sample and the reports are given very carefully to the patients that helps them for their further treatment.

One can also compare the results of CRP quantitative test as well as CRP semi-quantitative test to see the difference in the proportion of the values of both the results and also to analyze the positivity of the patient's sample in the laboratory.

CRP semi-quantitative test is very similar to the CRP latex test as it can also be done on the six circle slides but, firstly it have to be loaded with normal saline water and mix it up with the same quantity of the sample and add the reagents as the analyzer do into the latex test and rotate it for 2-3 minutes to get the appropriate result.

The results are seen into the decreasing order as the agglutination in first circle is always greater than the second one and the agglutination of the second circle in second circle is more than the third one. So, the results are counted in the form of 1:2, 1:4, 1:8 and so on.

CRP semi-quantitative test is commonly known as dilution test also because in this test the analyzer mixes the sample with the normal saline solution and dilute it with the sample reagent and then mixes it properly to get the proper result of the test.

This test is done to conform the positivity of the test as sometimes analyzer feel a little doubt in the result but if the positivity comes into the dilution then the result can be given based on this test and thus this is very well known conformatory test of the serology lab.

These tests are mostly performed into the serology laboratory as these tests are performed by the separation of blood serum from the collected blood samples which are centrifuged later to separate the serum from the blood samples tand then we are able to perform each and every test of CRP.

CRP tests are done collectively altogether at the same time to conform the test that is either the test is Latex or Quantitative or it is Semi-Quantitative, one can do the all tests together at the same time and also from the same sample and one can be able to

know the difference in the values of the each and every test and then analyze their results collectively.

### **CRP Test Results :-**

The a high level of CRP , shows it probably means there is some type of inflammation in the body. A higher than normal CRP level does not necessarily mean that there is a medical condition needing treatment, there are other factors also that can raise CRP levels such as smoking, obesity and lack of exercise.

In latex test, the CRP positive results as an agglutination on the card's circle surface.

In quantitative test, the CRP results as a graphical representation both in the positive or negative resulting.

In semi-quantitative result, the CRP results as an agglutination on card's each circle to where the sample is being loaded but in higher to lower level of agglutination of the CRP.

- ✓ A CRP test is sometimes get confused with a hs-CRP or High Sensitivity CRP test, they are used to diagnose different conditions. An hs-CRP test measures much lower levels of CRP. It is used to check the risk of heart diseases.
  
- The CRP test is often performed with another blood test called the Erythrocyte Sedimentation Rate (ESR). Both are non-specific markers for inflammation but, together, can offer important clues as to what is going on in the body.
- The main difference between the two tests is that changes occur more quickly with CRP. For instance, CRP may drop to normal levels quickly once an infection is cleared, while ESR will remain elevated. In such cases, the ESR can help to reveal the footprint of an illness even as the symptoms resolve.
  
- ❖ There is also a hs-CRP test that measures very low amounts of CRP in order to predict a person's risk of heart attack and stroke.
- ❖ The CRP test is a non-specific blood test used to measure levels of C-reactive protein, a marker of inflammation. Based on how much CRP levels are raised, a healthcare provider can narrow the possible causes of an illness.

High-sensitivity assays, such as nephelometric assays, are used to detect the baseline levels of CRP and patients who are at risk of cardiovascular disease. An individual with a CRP level more than 3mg./L has an increased risk of coronary heart disease, and this risk increases in those with type 2 diabetes.

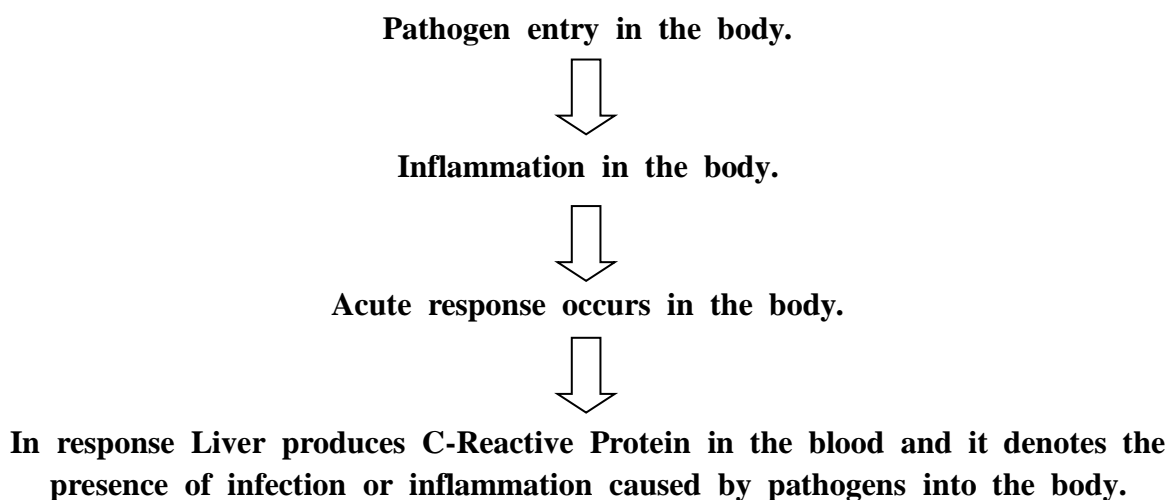
Although studies have shown that CRP levels increase during infections and inflammatory diseases, the precise role of CRP isoforms in their development and progression remains largely unknown. Thus, urgent investigations are required to determine the effects of each CRP isoforms on specific cellular processes during disease development.

CRP levels are known to increase dramatically in response to injury, infection and inflammation. CRP is mainly classed as an acute marker of inflammation, but research is starting to indicate important roles that CRP plays in inflammation.

CRP is mainly classed as an acute marker of inflammation, but research is starting to indicate important roles that CRP plays in inflammation. CRP is the principal downstream mediator of acute-phase response following an inflammatory event and is primarily synthesized by IL-6 dependent hepatic biosynthesis.

The main role of CRP in inflammation leads to focus around the triggering of the C1q molecule in the complement pathway leading to the opsonization of pathogens.

A flowchart that follows the production of CRP in the body is –



- ✓ Both CRP quantitative and semi-quantitative tests are done to analyze the proper amount of CRP present in the blood serum and thus these two methods are more acceptable methods of the CRP and thus they are mostly performed methods.
- ✓ Both methods are done very effectively and with great concern and thus they are done very carefully to know the correct result of the CRP in the blood that is produced by the liver due to some kind of infection and inflammation in the body due to any kind of medication, surgery or there is some kind of disease in the body results into the production of CRP into the bloodstream which is checked out by serum separated from the collected blood samples present into the laboratory.

CRP increases upto 1000 fold at the site of infection or inflammation in the body of the infected person. Some evidences suggest that estrogen in the form of hormone replacement therapy influences CRP levels in the elderly.

CRP was discovered while investigating the sera of patients suffering from the acute stage of Pneumococcus.

There are many factors that can alter baseline CRP levels including age, gender, smoking status, weight, lipid levels and blood pressure. The average levels of CRP in serum in a healthy individual is around 0.8mg/L, but this baseline can vary greatly in individuals due to other factors.

- C-Reactive Protein is very important protein secretion in the body as it denotes various kinds of inflammations or infections or the presence of any kind of pathogens in the body.



## **AIM AND OBJECTIVES**

### **3. AIM AND OBJECTIVE**

**Aim of Project :-**

Comparative analysis of semi-quantitative CRP test using latex agglutination method and quantitative CRP using nephelometric method.

**Objective of Project :-**

Analyze the samples serum into the biochemistry analyzer by nephelometric method.

If the CRP level is high, analyze the sample serum into the slide by latex method.

Then, compare both the values and form the result.

## **REVIEW OF LITRETURE**

## **4. REVIEW OF LITRETURE**

C – Reactive macromolecule (CRP) is associate annulate pentameric macromolecule found in blood plasma, that has the current concentrations rise in response to inflammation. It is associate acute section macromolecule of internal organ origin that will increase following lymphokine – six secretion by macrophages and T – cells. Its physiological role is to bind to lysophosphatidylcholine showed on the surface of dead or dying cells and some sorts of microorganism in order to activate the complement system via C1q.

CRP is synthesized by the liver in response to factors free by macrophages and fat cells (adipocytes). It is a member of the pentraxin family of proteins. It is not interconnected to C – amide (insulin) or macromolecule C (blood coagulation). C – reactive macromolecule was the primary Pattern Recognition Receptor (PRP) to be known.

### **4.1 History of serum globulin :-**

Discovered by Tillett and Francis in 1930, it was primarily thought that serum globulin would possibly be a unhealthful secretion since it was accumulated in a selection of unhealthiness, together with cancer. The additional discovery of internal organ synthesis (made in the liver) indicated that it is a native macromolecule. Initially, serum globulin was measured victimization the prevention reaction that gave a positive or a negative result. additional precise strategies now-a-days use dynamic lightweight scattering when reaction with serum globulin – specific antibodies.

CRP was named thus as a result of it was initial recognized as a substance in the body fluid of patients with acute inflammation or infection that reacted with the cell wall saccharide (C – Polysaccharide) of *Diplococcus pneumoniae*.

### **4.2 Genetics and Structure :-**

The serum globulin cistron is gift on body – one. It is a member of the little pentraxins family. The compound has 224 amino acids, and molecular mass of twenty five,106 Da. The complete macromolecule, created up of 5 monomers, has a total mass of around one hundred twenty,000 Da. In serum, it gathers into stable pentameric structure with a discoidal form.

### **4.3 Functions :-**

CRP binds to the phosphocholine showed on the surface of microorganism cells such as *Diplococcus pneumoniae* microorganism. This enhances the complement system,

encouraging bodily function by macrophages, that clears death and apoptotic cells and microorganism. This is thus known as acute phase response that happens as a result of increasing concentrations of IL – six, that is made by macrophages as well as adipocytes in response to a huge variety of acute and chronic inflammatory conditions such as microorganism, infective agent or flora infections, rheumatic and different inflammatory diseases, malignancy and tissue injury and necrosis. These conditions cause release of lymphokine – six and different cytokines that trigger the synthesis of serum globulin and factor I by the liver.

CRP binds to phosphocholine on microorganisms. It is well thought to assist in complement binding to foreign and broken cells and enhances bodily function by macrophages, that categorically a receptor for serum globulin. It plays a major role in innate immunity as a primary defense system against infections.

- The main purpose of serum globulin check is the detection of inflammation in the body.
  - The check of serum globulin detects the quantity of serum globulin in the blood.
- In healthy adults, the traditional concentrations of serum globulin varies between zero.8 mg / L and three.0 mg / L. However, some healthy adults show accumulated serum globulin at ten mg / L. serum globulin concentrations conjointly increase with age, could be due to the subclinical conditions. There is conjointly no renowned variations of serum globulin concentrations. cistron polymorphism of lymphokine – one family, lymphokine – six and polymorphic GT repeat of the serum globulin cistron do have an effect on the traditional serum globulin concentrations once a person will not have any medical conditions. The plasma half-life of serum globulin is nineteen hours, and is constant in all medical conditions.
  - Once there is an incitation, the serum globulin level will increase ten,000 fold from less than fifty metric weight unit / L. Its concentration will increase to five mg / L by six hours and peak at forty eight hours. Therefore, the solely part that affects the blood serum globulin concentrations is its production rate, that will increase with inflammation, infection, trauma, necrosis, malignancy and allergic reactions.
  - In acute inflammation, serum globulin will increase as abundant as fifty to a hundred mg / L at intervals four to six hours in gentle to moderate inflammation or associated infection such as skin infection, urinary tract infection or respiratory illness. It will get double in every and each eight hours and reaches its peak increment at thirty six to fifty hours following injury or inflammation. serum globulin between a hundred to five hundred mg / L is thought of extremely diagnostic of inflammation due to microorganism infection.

- Once acute inflammation falls down, the serum globulin level falls quickly as a result of its comparatively short half-life. Serum globulin concentrations between a pair of and ten mg / L are thought of as metabolic inflammation, metabolic pathways that cause arterial sclerosis and kind II polygenic disease mellitus.

#### **4.4 Principle :-**

The serum globulin check is based mostly on the principle of the latex agglutination. Once latex particles complexed human opposing – serum globulin are mixed with a patient's body fluid containing C-reactive proteins, acute invisible agglutination reaction can take place at intervals a pair of minutes.

#### **4.5 Uses :-**

CRP could be used to discover or monitor important inflammation in acute degree individual WHO is suspected of having acute degree acute condition, such as serious microorganism infection like infection, a flora infection and acute Inflammatory malady (PID).

The CRP check is helpful in observing individuals with chronic inflammatory conditions to discover flare – ups or to confirm if treatment is effective.

The determination of the CRP level is helpful to monitor the medical aid.

It is done to check for infection when surgery. CRP levels commonly rise inside a pair of to half-dozen hours of surgery and then go down by the third day when surgery.

If CRP levels keep elevated three days when surgery, acute degree infection could be given.

#### **4.6 Limitations of CRP check :-**

- 1). The strength of the agglutination reaction is not indicative of the CRP concentration. Weak reactions could occur with slightly elevated or markedly elevated concentrations.
- 2). A prozone development (antigen excess) could cause false negatives. It is judicious, therefore, to check all negative sera by re – testing at a 1:10 dilution.
- 3). Reaction times longer than specific could turn out apparent false reactions due to a drying result.

- 4). powerfully lipemic or contaminated sera will cause false positive reactions.
- 5). solely liquid body substance ought to be used in this check.
- 6). A quantitative volumetric analysis procedure on positive specimens is needed to observe increasing or decreasing levels.
- 7). Patients with high titers of arthritic factors could offer positive results.

#### **4.7 CRP Quantitative take a look at :-**

CRP quantitative take a look at is a lot of sensitive, space responding indicator than ESR. C-reactive protein quantitative might be used to sight early post – operative wound infection and to follow therapeutic response to anti – inflammatory agents. C-reactive protein is associate active part chemical, that will be used as a take a look at for inflammatory diseases, infections and growth diseases. Progressive will increase correlate with will increase of inflammation / injury.

Recent reports have indicated that a extremely sensitive version of the C-reactive protein assay might be used as associate further indicator for status to viscus diseases.

Its limitations includes that C-reactive protein arises as a non – specific responses to tissue injury and inflammation.

CRP quantitative uses the methodology of nephelometric method to perform the take a look at.

#### **4.8 CRP Semi – Quantitative take a look at :-**

A semi – quantitative latex agglutination take a look at for bovine humor liquid body substance bodily fluid body fluid humor. C-reactive protein levels has been established by adding mixture diluted serum with a 1 Chronicles latex suspension containing zero.489 micrometer latex particles coated with affinity – pure protein at a quantitative relation of twenty micrograms / mg latex. The agglutination was performed on a glass slide in a wet chamber at space temperature with forty five min. incubation. This take a look at is reliable, consistent and the results correlate with those of the single radial immunodiffusion (SRID) take a look at. The impact of low temperature storage on C-reactive protein concentration disclosed a half-hour degradation of C-reactive protein throughout a pair of years storage at four degrees C. The attainable role of

EDTA addition to stop a decrease in humor C-reactive protein concentration by phase change and thawing is conjointly mentioned.

C-Reactive supermolecule is a very important characteristic acute part supermolecule in humans. Its short half-life makes it a significantly smart indicator to notice and follow any illness activity. The objective of this study was to compare semi-quantitative slide latex agglutination check with quantitative turbidimetric bioassay technique for detection of CRP. The sera of some patients clinically suspected to have general infection were tested victimization 2 ways. **(A comparative study of semi-quantitative latex agglutination check and quantitative turbidimetric bioassay technique for the detection of C-reactive supermolecule from human sera by Manisha N. Dhamecha, Mayurika K. Patel, Urvesh V. Shah.,2013)**

Serum C-reactive supermolecule is associate acute part indicator in humans that is terribly helpful for the diagnosing and analyzing of infectious illness. CRP measure additionally helps in differential diagnosing, in the management of baby blood disease and infectious disease wherever customary microbiological investigations ar terribly a lot of time taking. machine-driven particle increased turbidimetric bioassay ar higher for routine analysis of human liquid body substance CRP levels. The objective of this study is to judge whether or not human CRP levels might be measured by latex agglutination slide technique and compared with particle increased turbidimetric bioassay. **(Comparison of a fast semi-quantitative latex agglutination slide technique against quantitative particle increased turbidimetric bioassay for measure of C-reactive supermolecule by Riyaben Trivedi, Abdul Rehman Amer, Riddhi Patel, Pradip Trivedi.,2019)**

C-reactive supermolecule is associate acute part supermolecule made in liver. It is less than five mg/dL in the blood liquid body substance and alternative body fluids of traditional people, however it is inflated suddenly among a few hours following infectious reactions. The objective of this study was to look over CRP level by qualitative and quantitative ways. measure of C-reactive supermolecule by each the ways is used for designation and watching general inflammatory illness. The quantitative technique by neflometre is done by scrutiny its studies with semi-quantitative technique by latex technique. **(Comparison of qualitative and quantitative study of c-reactive supermolecule by Mahomet Reza Kiaei, Mahomet Hedayat Mofidi, Faramarz Koohsar, Abolfazl Amini.,2014)**

The study evaluated a new fast semi-quantitative immunometric assay of C-reactive supermolecule as a screening check for infection by comparison with associate machine-driven nephelometric technique. The study measured C-reactive supermolecule by the semi-quantitative technique and compared the results with those obtained with a neflometer by



quantitative technique. Semi-quantitative technique is somewhere a half of latex technique used for the detection of CRP analysis. **(Rapid and semi-quantitative assay of C-reactive supermolecule evaluated and compared by Hilary D. Vallance, Gillian Lockitch.,1991)**

C-reactive supermolecule is acute part supermolecule, wide used in primary diagnosing of baby infection. This study provides associate outlet of comparison of a fast purpose of care semi-quantitative card check was done with quantitative check for measure of C-reactive supermolecule in few suspected cases of baby infection. The study ascertained terribly high correlation between 2 ways in traditional as well as abnormal samples. The study additionally ascertained that semi-quantitative fast check provide ease of use and interpretation and thence ideal for doctors. **(Comparison of fast semi-quantitative card check against immunoturbidimetric quantitative check for determination of C-reactive supermolecule levels in baby infection by P.R. Naik, S.S. Naik, S.B. Bharadwaj, P.B. Desai.,2013)**

A key issue behind the unneeded use of antibiotics is the lack of fast and correct diagnostic tests. In this study, one determines 2 novel ways to discover C-reactive proteins connected infections among few minutes by victimization the neflometric and latex technique one once the alternative to grasp the distinction between the results of each quantitative and semi-quantitative technique to analyze the level of inflammation in one's body. **(Rapid detection of inflammation victimization novel ways quantitative and semi-quantitative C-reactive supermolecule in patients body by Jari Nuutila, Ulla Hohenthal, Jarmo Oksi, Paivi Jalava.,2021)**

Having each polygenic disease associated an elevated CRP (CRP) level compounds one's risk of developing disorder, that folks with polygenic disease are at specific high risk. CRP is each a biomarker for infectious and non-infectious disorders related to inflammation and a risk issue for such conditions. several researchers currently believe that the supermolecule additionally plays a job within the illness processes. **(C-Reactive supermolecule and disorder in folks with polygenic disease by principle Xu, Kyra Whitmer.,2006)**

Synovial CRP testing wasn't a lot of discriminating than CRP within the diagnosing of hip and knee periprosthetic joint infection (PJI). A liquid body substance CRP level larger than 9mg/L was an indication of PJI. The diagnosing of PJI may be difficult and rests on many principles. the utilization of diagnostic biomarkers, like the secretion CRP. **(Comparative worth of corpuscle erythrocyte sedimentation rate (ESR) and CRP (CRP) Testing together versus on an individual basis for the diagnosing of uniform Patients by Nazila Assasi, Gord Blackhouse, Kaitryn Campbell, Robert B.Hopkins, Mitchell Levine.,2015)**

Point-of-Care CRP testing is probably going to produce an economical diagnostic intervention each in terms of reducing antibiotic prescribing and in terms of quality-adjusted life years gained. **(Cost Effectiveness of CRP Testing to tell Antibiotic prescribing selections by Raymond Oppong et al., 2013)**

C-Reactive supermolecule is a very important characteristic acute part supermolecule in humans. Its short half-life makes it a significantly smart indicator to notice and follow any illness activity. The objective of this study was to compare semi-quantitative slide latex agglutination check with quantitative turbidimetric bioassay technique for detection of CRP. The sera of some patients clinically suspected to have general infection were tested victimization 2 ways. **(A comparative study of semi-quantitative latex agglutination check and quantitative neflometric bioassay technique for the detection of C-reactive supermolecule from human sera by Manisha N. Dhamecha, Mayurika K. Patel, Urvesh V. Shah.,2013)**

A methodology for the determination of CRP by latex immunochemical assay that measures the increase in optical density as a result of latex agglutination due to matter – protein reaction. This latex agglutination mensuration immunochemical assay will be used for quantitative analysis of numerous biological substances. moreover, the usual methodology used in the clinical microprecipitation methodology, has the lower detection limit of some ten mg / L. the methodology conferred here is adequately sensitive to live the low concentration of CRP among healthy people that has not been potential except by victimization RIA. **(A new immune quantitative methodology by latex agglutination application for the determination of humor C – Reactive macromolecule (CRP) and its clinical significance by Senju et al. and J. Clin.,1986)**

## **MATERIALS AND METHODS**

## **5. MATERIALS AND METHODS**

- **Materials Used :-**

**5.1). RHELAX-CRP Latex Reagent Kit –**

➤ **Principle of Kit**

RHELAX-CRP slide check for detection of serum globulin is primarily based on the principle of agglutination. The check specimen that is blood serum is mixed with RHELAX-CRP latex chemical agent and allowed to react.



**Fig. 5.1 : Kit used to perform Semi – Quantitative Test**

If serum globulin concentration is bigger than 6 mg / L a visible agglutination is ascertained. If serum globulin concentration is less than 6 mg / L, then no agglutination is ascertained.

Materials provided with this kit are –

i). REAGENTS :

- a). RHELAX-CRP Latex Reagent
- b). Positive Control
- c). Negative Control

ii). ACCESSORIES :

- a). Slide with Six Reaction Circles
- b). Sample Dispensing Pipettes
- c). Mixing Sticks
- d). Rubber Teat

Additional materials required in laboratory for CRP test are –

- i). Stop Watch
- ii). High Intensity Direct Light Source
- iii). Isotonic Saline

## 5.2). CRP Turbilatex Beacon Kit –

### ➤ Principle of Kit

CRP turbilatex is a quantitative turbidimetric check for the measure of C-reactive super molecule (CRP) in human blood serum or plasma. Latex particle coated with specific anti-human serum globulin area unit agglutinated once mixed with samples containing serum globulin.



**Fig. 5.2 : Kit used to perform Quantitative Test**

The agglutination causes an absorbance modification dependent up on the serum globulin contents of the patient sample that will be quantified by comparison from a calibrator of famed concentration.

Materials provide with this kit are –

i). REAGENTS :

- a). Reagent 1 – Diluent
- b). Reagent 2 – Latex Antigen
- c). Reagent 3 – CRP Calibrator

Additional materials required in laboratory for CRP test are –

- a). Clean and Dry Glassware
- b). Pipettes, Tips and Micropipettes
- c). Biochemistry Analyzer
- d). Vortex Shaker

**5.3). ). Other used Materials that are not provided with the Kits –**

- Normal Saline
- Distilled Water
- Vortex Shaker
- Dancing Shaker
- Pipettes (1000 microgram / L, 200 microgram / L, 10 microgram / L)



**Fig. 5.3 : Materials and Instruments used in CRP Testing**

#### **5.4). Semi-Auto Biochemistry Analyzer –**

##### **➤ Principle**

A semi-automatic organic chemistry instrument follows the quantitative chemical analysis, measurement and absorbance principles for operating underneath the optical techniques, whereas it follows the operating of direct potentiometry and indirect potentiometry principles underneath the class of chemistry techniques.

A machine necessary for doing blood tests such as aldohexose, urea, albumin, etc. It depends on the principle of filter measurement. It contains a pump to sip the sample and the distilled water into the machine, and brooder to hold some check tubes in it as well as an intrinsic thermal printer at the high to print the results from the machine.

It is a chemical instrument machine that measures the parts among a collected biological sample consisting of blood, urine, plasma and therefore on. This machine permits the designation of diseases and the potential cause of problems and symptoms in the human body.



**Fig. 5.4 : Semi – Auto Biochemistry Analyzer**

This analyzer's work primarily based on 2 measure strategies : Optical Techniques and chemistry Techniques.

These analyzers area unit a lot of sensible for use in smaller laboratories and medical practices. This is due to it being ready to handle a lower range of samples at a time as compared to the totally automatic one.

For this, the samples and reagents area unit not pre – ready and hold on however set up singly every time a check is conducted. That is why it is Semi – Automatic, although this slows the method of testing as a whole it has a nice profit as it provides tremendous flexibility once the sort of tests differs creating the reagents vary every time.



- **Methods Used** :-

The basic method used in the laboratory to compare the values of quantitative and semi-quantitative techniques are as follows –

Collect the blood samples in the plain vials from patients and bring them to the room temperature in the laboratory.



Bring the reagents also at the room temperature to analyze perfect results.



Centrifuge the plain vials at 4000rpm for 2 minutes.



Take eppendrofs according to the number of tests of the given samples.



Then, add 450 microlitre diluents and 50 microlitre latex antigen into the eppendrof by using the pipettes of 1000 microlitre and 200 microlitre, respectively.



Vortex the eppendrofs for a while for the proper mixing of the reagents.



Prepare the semi-auto analyzer to test the CRP from the blood serum.



Wash the machine with distilled water by using sipper present onto the machine, then give a blank test before the sample.



Then, add 5 microlitre of serum sample into the eppendrof and mix it properly and carefully.



Now, vortex the whole mixture again for the proper mixing of the sample with the reagents into the eppendorf.



Now, place the eppendorf to the sipper tube and press the button to allow the machine to suck the sample in it.



Then, wait for the result for 2 minutes that means 125 seconds for the appearance of the graph onto the screen.



Now, the result is positive the machine shows value that is more than 6mg/L with graphical representation on the screen.



Now, take slides with six reaction circles and dispense a 50 microlitre of normal saline in each circle and dispense a single-single drop of same sample in each circle onto the slide.



Add small drop of CRP reagent to each circle of dispensed sample by its dropper.



Mix the sample, normal saline and the reagent carefully by mixing sticks one by one with separate sticks for separated sample circle.



Place the cards on the dancing shaker for 2 minutes to get the appropriate results from the samples.



The result shows agglutination in each circle one after the other and one less than before one of each slide.



Approx 2,3 or 4 circles will show agglutination depending upon positivity of quantitative or qualitative test.



The value is written in the form of 1:2, 1:4, 1:8, 1:16, 1:32 and so on.

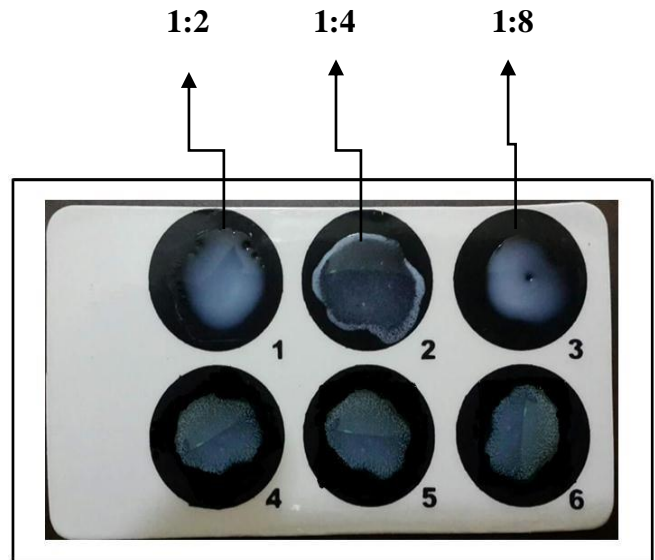


Calculation can be done by using following formula :-

$$\text{CRP (mg/L)} = S * D$$

Where, S = sensitivity of the reagent that is 6mg/L

D = highest dilution of serum showing agglutination.



**Fig. 5.5 : Comparison of Quantitative and Semi – Quantitative Results**

There are total three main and major methods that are commonly used in the laboratory to perform CRP test, they are-

2). Rapid Card Test –

Collect the blood samples in the red top tubes from patients and bring them to the room temperature in the laboratory.



Bring the reagents also at the room temperature to analyze perfect results.



Centrifuge the tubes at 4000rpm for 2 minutes.



Take slides with six reaction circles and dispense a single-single drop of sample in each circle with the positive and negative controls on first and second circle by using sample dispensing tubes with rubber teat.



Add small drop of CRP reagent to each dispensed sample by its dropper.



Mix the sample and the reagent carefully by mixing sticks one by one with separate sticks for separated samples and controls.



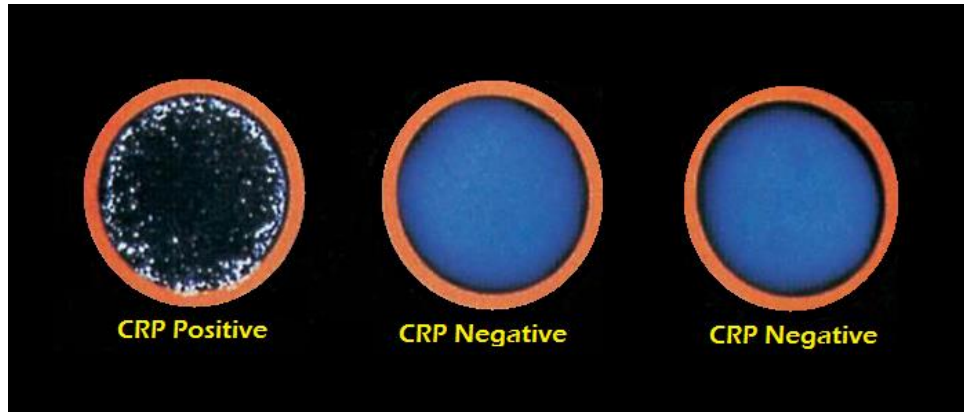
Place the cards on the dancing shaker for 2 minutes to get the appropriate results from the samples.



If the result is positive, the sample shows agglutination on the outer surface of the mixed circles with the presence of granules into the sample.



If the result is negative, the sample does not show any agglutination and remains same.



**Fig. 5.6 : Positive and Negative Results on Latex Method**

### 3). Quantitative Test –

Collect the blood samples in plain vials from patients and bring them to the room temperature in the laboratory.



Centrifuge the plain vials at 4000rpm for 2 minutes.



Take eppendrofs according to the number of tests of the given samples.



Then, add 450 microlitre diluents and 50 microlitre latex antigen into the eppendorf by using the pipettes of 1000 microlitre and 200 microlitre, respectively.



Vortex the eppendrofs for a while for the proper mixing of the reagents.



Prepare the semi-auto analyzer to test the CRP from the blood serum.



Wash the machine with distilled water by using sipper present onto the machine, then give a blank test before the sample.



Then, add 5 microlitre of serum sample into the eppendorf and mix it properly and carefully.



Now, vortex the whole mixture again for the proper mixing of the sample with the reagents into the eppendorf.



Now, place the eppendorf to the sipper tube and press the button to allow the machine to suck the sample in it.



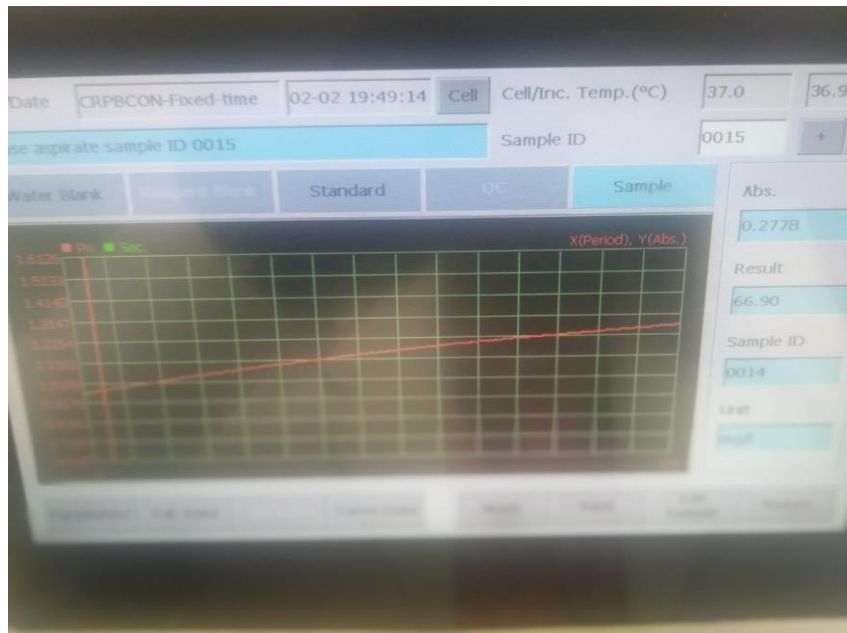
Then, wait for the result for 2 minutes that means 125 seconds for the appearance of the graph onto the screen.



If the result is positive the machine shows value that is more than 6mg/L with graphical representation on the screen.



If the result is negative the machine shows value that is less than 6mg/L with graphical representation on the screen.



**Fig. 5.7 : Graph showing Positive Result on Analyzer Screen**

**4). Semi-Quantitative Test –**

Collect the blood samples in red top tube from patients and bring them to the room temperature in the laboratory.



Centrifuge the tubes at 4000rpm for 2 minutes.



Take slides with six reaction circles and dispense a 50 microlitre of normal saline in each circle and dispense a single-single drop of same sample in each circle onto the slide.



Add small drop of CRP reagent to each circle of dispensed sample by its dropper.



Mix the sample, normal saline and the reagent carefully by mixing sticks one by one with separate sticks for separated sample circle.



Place the cards on the dancing shaker for 2 minutes to get the appropriate results from the samples.



The result shows agglutination in each circle one after the other and one less than before one of each slide.



Approx 2,3 or 4 circles will show agglutination depending upon positivity of quantitative or qualitative test.



The value will be written in the form of 1:2, 1:4, 1:8, 1:16, 1:32 and so on.

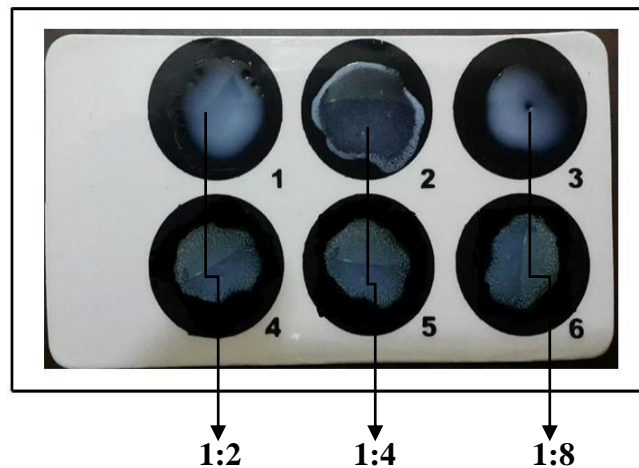


Calculation can be done by using following formula :-

$$\text{CRP (mg/L)} = S * D$$

Where, S = sensitivity of the reagent that is 6mg/L

D = highest dilution of serum showing agglutination.



**Fig. 5.8 : Ratio on Slide Circles by Latex Agglutination Method**



- Semi-quantitative test is done to know the correct amount and ratio of positive CRP qualitative or quantitative test.
- Quantitative test is done to know the exact amount of positive or negative CRP test.
- Qualitative test is used only to know the positivity or negativity of CRP test.



**Fig. 5.9 : Samples used into the Comparative**

## **RESULT AND DISCUSSION**

## **6. RESULT AND DISCUSSION**

- **Result :-**

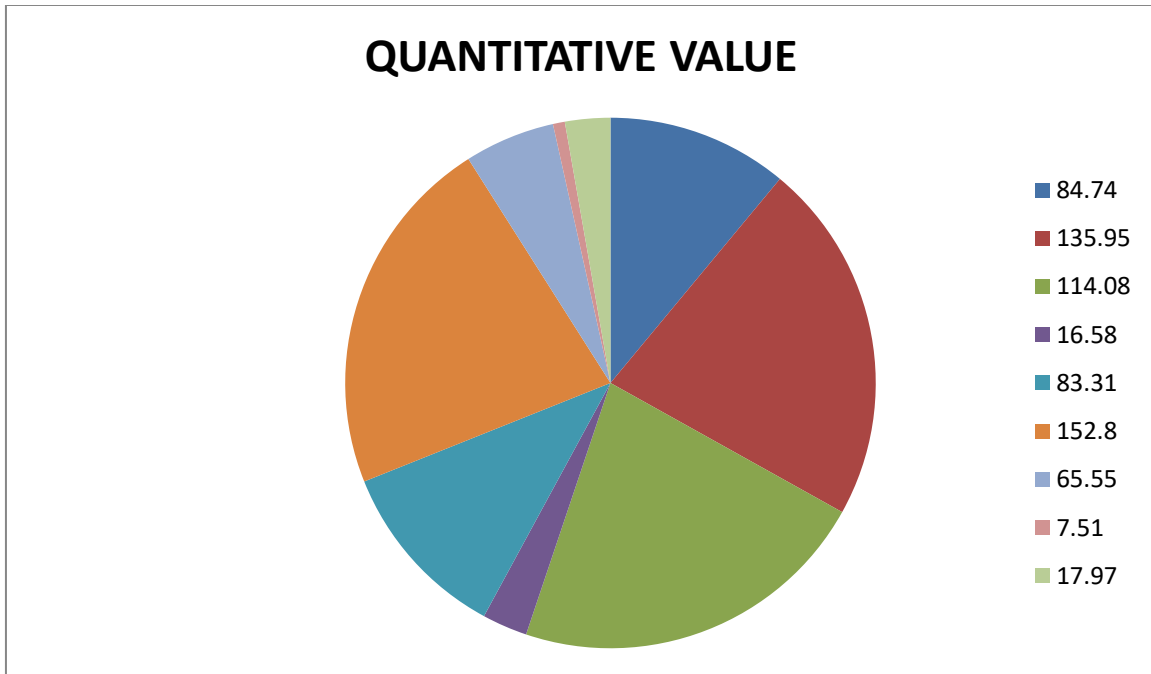
The result of this project is dependent on Prospective and Observational Study.

The result is given in the form of table as shown below –

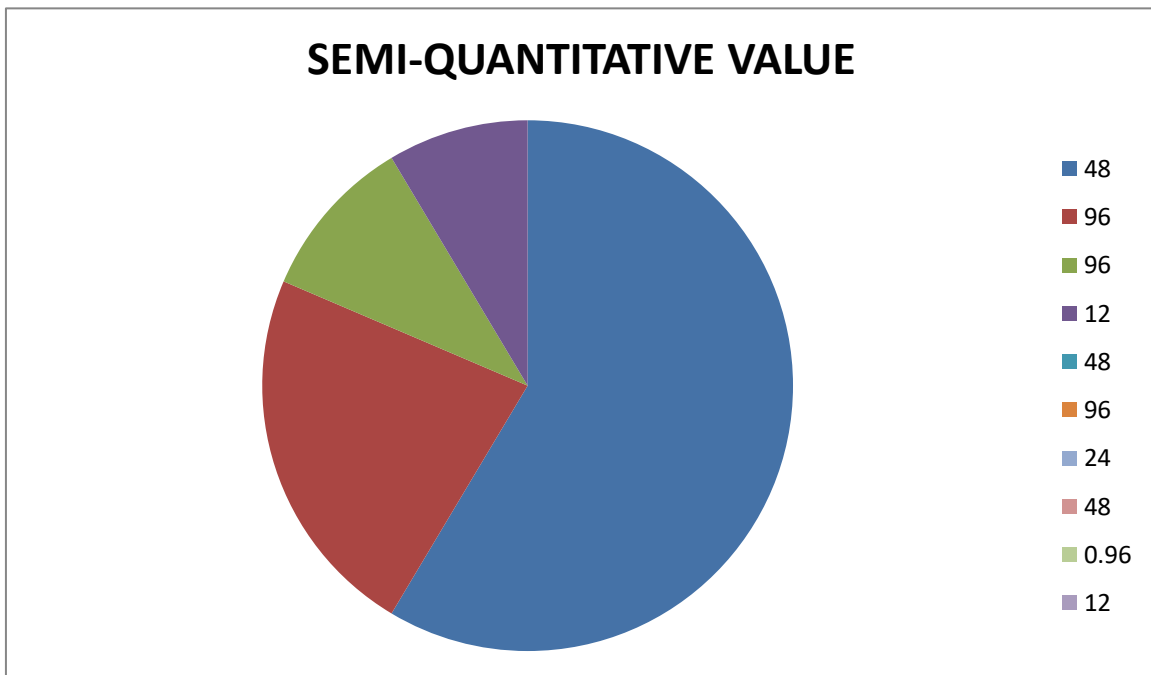
| S. No | Date of Collection | CR Number of Patients | Age of Patients | Sex of Patients | Quantitative Value | Semi – Quantitative Value |
|-------|--------------------|-----------------------|-----------------|-----------------|--------------------|---------------------------|
| 1.    | 21/03/22           | 115494                | 44              | M               | 84.74 mg/L         | 1:8 = 48 mg/L             |
| 2.    | 22/03/22           | 004706                | 51              | F               | 135.95 mg/L        | 1:16 = 96 mg/L            |
| 3.    | 25/03/22           | 004960                | 52              | F               | 114.08 mg/L        | 1:16 = 96 mg/L            |
| 4.    | 26/03/22           | 007379                | 70              | F               | 16.58 mg/L         | 1:2 = 12 mg/L             |
| 5.    | 30/03/22           | 136328                | 21              | M               | 83.31 mg/L         | 1:8 = 48 mg/L             |
| 6.    | 08/04/22           | 006659                | 82              | F               | 152.80 mg/L        | 1:16 = 96 mg/L            |
| 7.    | 16/04/22           | 008104                | 42              | F               | 65.55 mg/L         | 1:4 = 24 mg/L             |
| 8.    | 18/04/22           | 005514                | 29              | F               | 82.84 mg/L         | 1:8 = 48 mg/L             |

|     |          |        |    |   |            |               |
|-----|----------|--------|----|---|------------|---------------|
| 9.  | 21/04/22 | 008485 | 28 | F | 7.51 mg/L  | No Result     |
| 10. | 26/04/22 | 016602 | 45 | M | 17.97 mg/L | 1:2 = 12 mg/L |

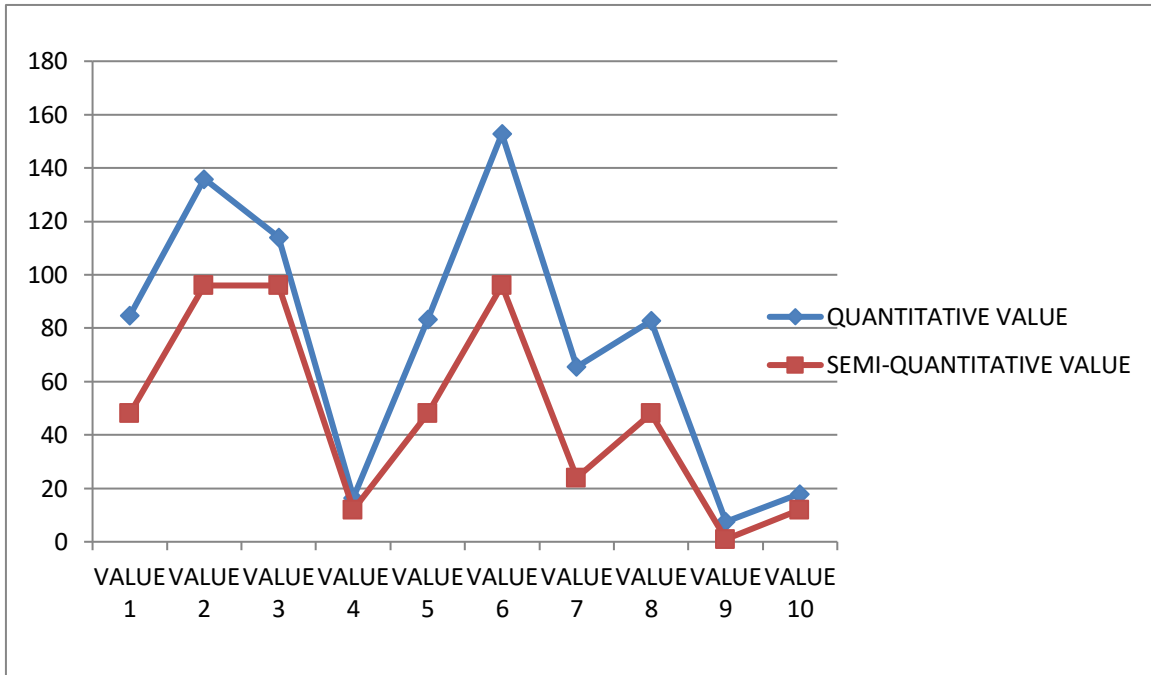
**Table - 6.1**



**Fig. 6.1: Pie Chart showing Variations in the Quantitative Values of Samples**



**Fig. 6.2 : Pie Chart showing Semi-Quantitative Values of Samples on comparing values with the Quantitative Results**



**Fig. 6.3 : Line and Dotted Line Chart showing the similarities in Quantitative and Semi-Quantitative Values of the Sample**

By the help of this line chart one can also observe that the comparative values of both the results are very close to each other.

Here, different colored lines shows the values of quantitative as well as semi-quantitative results that shows the simultaneous fluctuations in the values of the sample.

Mode Value of Semi-Quantitative Value is as follows –

$$\text{Mode} = 96 \text{ and } 48.$$

Mode Value of Quantitative Value is as follows –

There is no repetitive value in the quantitative results as most of the time the quantitative results

fluctuate every time one sucks up the sample into the machine.

The statistical representation shows that the mean value of quantitative results are higher than the comparison of semi-quantitative value which represents that the quantitative values are higher than the semi-quantitative values and thus one consider quantitative value as over the semi-quantitative value because it gives the exact value of the sample whereas in semi-quantitative value one consider the result that depends on the reactivity on the circles of the reaction slides whereas the semi-quantitative values contain some repetitive values but the quantitative results very rarely contain any repetitive value in its each and every result.

Here, the analyzer performed 10 CRP tests by both quantitative and semi-quantitative method. Of 10 CRP test results that were  $>6$  mg/L by the comparison method, all were read correctly by semi-quantitative method that were  $>12$  mg /L with rare false negative.

From all CRP results, 9 of 10 were correctly read as  $>12$  mg /L by semi-quantitative method but 1 of 10 does not show any result in the semi-quantitative method but it shows the sample positivity into the quantitative method due to the sensitivity of the reagents as well as machine.

Of 10 tests the tests that shows its results between 48 and 96 mg /L by semi-quantitative method, 6 would have been placed in a higher category by semi-quantitative tests. However, all would still have been interpreted as true positive, and the clinical management would probably not have been altered.

- **Discussion :-**

According to this study, analyzer found that the difference between the values of the quantitative and semi – quantitative values are bit high as compared to the study of **Manisha N. Dhamecha, Mayurika K. Patel, Urvesh V. Shah** on the same comparative study the analyzer have concluded that this project shows the correct amount of difference between the comparative values of both the methods. So, it concluded that result shown in this project is correct.

According to this study, analyzer found that the values of CRP is dependent upon the age of the patient as compared to the study of **Senju et al. and J. Clin** on the same comparative study of the people with young and old age patient that shows the same result of higher CRP in old age. So, it concluded that the result shown in this project is correct.

According to this study, analyzer found that the increment in the value of CRP is mainly dependent on the presence of any kind of disease in the patient's body as compared to the study of **Xu, Kyra Whitmer** on the same comparative study between healthy and diseased person into a community which shows that sometimes healthy person also have a little rise in the value of CRP but in the diseased person the value of CRP is sometimes little and sometimes a little more higher than the normal value. So, it concluded that the result shown in this project that sometimes the values might differ as compared to the diseased and healthy person.

According to this study, analyzer found that the CRP value does not depend upon the gender of the patient as compared to the study of **P.R. Naik, S.S. Naik, S.B. Bharadwaj, P.B. Desai** on the same comparative study between male and female patients which shows that the increase or decrease in the CRP values does not depend on the gender of the patient. So, it concluded that the result shown in this project is correct.

According to this study, analyzer found that CRP results by nephelometric method is more sensitive than compared to the results by latex method as compared to the study of **Jari Nuutila, Ulla Hohenthal, Jarmo Oksi, Paivi Jalava** on the same comparative study of both these methods shows that the results are dependent on the sensitivity of the biochemistry analyzer it shows that the CRP results may vary in the positive and negative results into the patients. So, it concluded that the result shown on the 9<sup>th</sup> number represents that it shows a positive result by the nephelometric method whereas it shows a negative result by the latex method.



CRP measure in the given term provides the specific results that has been shown to be extremely sensitive and specific for serious microorganism infection, serum globulin conviction is terribly helpful in either supporting or disclaim the designation of infections or inflammations in patient's body.

Most of the gift quantitative tests of serum globulin, though designed with some machine-driven instruments, don't seem to be sensible or economical to run as single tests into the machine. Thus, it is troublesome to offer a fast turnaround time into the quantitative check.

The semi-quantitative check permits results to be obtained at intervals minutes of receiving a specimen, creating it associate ideal check for screening. The sensitivity of quantitative serum globulin technique in these samples was 100 percent at half dozen mg/L. This makes the check system accessible and prepared for the screening check, wherever the negative result at < half dozen mg/L will not permits the presence of any infections.

However, it is necessary to note that, though serum globulin begins to increase speedily once the onset of infection, it could not reach half dozen mg/L for as long as twenty four hours. Therefore, associate incubated low concentration of serum globulin at the time of clinical declination will not rule out infection, once 2 serum globulin values apart a < half dozen mg/L, the analysis of infection is extremely unlikely.

There will not seem to be any loss in sensitivity with decrease in physiological state age instrument found that serum globulin values between forty eight and ninety six mg/L by the comparison technique were of overestimated and browse as eighty-two hundred mg/L by quantitative technique. Analyzer recommend that results in the eighty-two hundred mg/L vary will be interpretend as equivocal and followed up twelve-twenty four hour later with repeat comparative testing. In this study, eighty nothing of the results were in equivocal class.

A specificity of 100 percent and a sensitivity of eighty nothing can be achieved with results > ten mg/L. Correlation between the 2 ways was smart once the serum globulin correlation was > ten mg/L by the comparison technique. Overall, the instrument found a slight tendency to overestimate serum globulin values by the semi-quantitative technique. As a smart response to opposing-microorganism medical care is associated with a speedy decline in serum globulin concentrations, quantitative serum globulin check could so be terribly helpful in analyzing infections or any kind of inflammations throughout any treatment.

Changes ascertained in the comparison of mean and mode serum globulin concentrations as determined, applied mathematics analysis discovered that the serum globulin level changes according to their ways of check. Therefore, one could conclude that throughout the crucial health reaction onset amount, serum globulin concentration was subject to a slight to extremely modification and additionally will go

on the far side high limits from the traditional vary.

However, fluctuations in the serum globulin concentrations will go on the far side the limits from the traditional vary that a clinically insignificant and therefore helps the doctors to confirm any health deteriorations in the patient's body.

Numerous authors purpose to the role of cytokines in the induced expression of acute – section proteins whereas light their importance for clinical nosology. The role of serum globulin being a marker of varied pathological conditions is the subject of a significantly careful analysis. In the case of health deteriorate reaction, despite numerous clinical symptoms resembling those of associate inflammation, the system will not fight any foreign biological materials.

The serum globulin is most likely the best, despite non-specific organic chemistry marker of pathological processes, principally those of inflammatory character. Thanks to its high speed and comparatively low price, determination of serum globulin levels provides a quick technique for the designation and the assessment of the stage, the extent, and the dynamics of changes of s pathological condition.

## **CONCLUSION**

## **7. CONCLUSION**

CRP is a protein made by one's liver. It's sent into one's bloodstream in response to inflammation. Inflammation is the body's way of protecting body tissues if the person have been injured or have any infection. It can generally cause pain, redness and swelling in any injured or affected area.

CRP test can be done in the serology laboratory in three different ways that are Latex Method, Quantitative Method as well as Semi – Quantitative Method.

In this study analyzer have compared the two different methods of CRP testing that is quantitative and semi – quantitative method in which the analyzer have seen that both the results are comparatively very near about to each other. The results of the quantitative methods are given directly by the nephelometric method by using biochemistry analyzer whereas the results of the semi – quantitative methods are given in the form of ratios by the dilution method by using six reaction slides and dancing shaker.

In this study, the analyzer has randomly chosen 10 patients that have positive CRP test results firstly from the biochemistry analyzer and then by the dilution method to analyze its result in the form of ratio. By the help of this study, the analyzer have observed that the value of ratio and the quantitative value comes very near to each other.

In this study, the analyzer has collected the sample from the sampling of the months of March and April and then analyze the samples from both the methods carefully by obtaining the results one after the other, simultaneously.

Here, the analyzer concluded that the quantitative method is much better than the semi – quantitative method because this method gives an exact quantity of CRP protein in the bloodstream and thus it represents the amount of inflammation in the person's body which helps the doctor to treat the patient carefully and easily.

CRP helps the doctor to analyze the patient's condition before and after the surgery or some other kind of medications as well, so as the level of CRP increases in the bloodstream it results in some kind of disease in patient's body and its test results helps the doctor to treat the patient well.

The results that are collected are above 6 mg / L in quantitative test but above 12 mg / L in semi – quantitative method as below 12 mg / L the semi – quantitative or dilution method does not show any kind of result on the reaction slides with the normal saline and the serum sample. So, the minimal positivity values of both the methods are different and thus the reaction cycle of both the methods are very different as one have to calculate the values of semi – quantitative test but the values of quantitative are very much exactly shown on screen by the help of graphical representation.

The result and discussion of this study includes the pie charts that represents the values of both the methods separately and thus shows the amount of repetitive values in the given table, with these two pie charts there is also a representation of line and dotted line graphs that shows the similarities between both quantitative and semi – quantitative values in the table.

So, the analyzer concluded from this study that the CRP test can help the physician to treat the patient by both kinds of methods appropriately as both kinds of methods gives an exact result of amount of CRP present into the bloodstream of patient's body.

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