

# [Measuring c3 concentration](https://assignbuster.com/measuring-c3-concentration/)

Complement system is a vital part of the immune system and its function can be determined by measuring the concentrations of the complement components. The term complement refers to a group of cell membrane proteins and plasma that plays a major role in the defence process of the host. One of the major proteins involved in the complement system is the C3 which along with other proteins initiate vasodilation at the site of inflammation (Parslow et al., 2001). C3 in activated by the C3 convertase of the classical pathway (one of the pathways of the complement system).

The compounds of the complement system are involved in two pathways which assembles the membrane attack complex onto a bacteria membrane. This leads to phagocytosis and lysis of the bacterial cells as well as the activation of an inflammatory response (North, 2010). C3 is a type of complement which is a cytokine- inducible acute- phase protein involved in the alternative pathway and is an important marker for inflammation (Koistine et al., 1999; Széplaki et al., 2004). C3 is found in the circulation at low concentrations (North, 2010).

Measuring the amounts of C3 and C4 leads to useful information in the diagnosis of a certain conditions for example systemic lupus erythmatosis (North, 2010). A lower concentration of the complement refer to the consumption of the complement in immune complex diseases (Koelle, et al., 1982). A high concentration of the complement is linked with body weight of the patient, as well as the glucose and lipid metabolism (Koistine et al., 1999). Several studies indicated that the high level of C3 is due to the complications caused by atherosclerosis (Széplaki et al., 2004). Patients serum components C3 as well as C4 are used to indicate complement consumption immune complex diseases (Mancini et al., 2003).

In this experiment the concentration of C3 in human serum was measured by using single radial immunodiffusion and the value was compared to the normal reference range. The normal reference range provided in this experiment was 400 µg/ ml. The advantage of using single radial immunodiffusion is that they offer the unique opportunity of quantifying antigens and antisera with great ease ( ). Anti- C3 antibodies were employed to the agar gel on an acetate strip of Gel- Bond. The wells were formed in the gel and were filled with standard serum samples which contained known concentrations of C3 complement. During incubation a precipitate of antigen- antibody complexes is were formed by the diffusion of C3 from the wells. A disc of precipitate was formed and its diameter was directly proportional to C3 concentration. The size of the disc of precipitate has a linear relationship with the initial concentration know C3 concentrations of the standards. Standard calibration curve was constructed to measure the C3 concentration of reference and test sera. The aim of this experiment was to measure the C3 concentration in the test serum sample and to access the accuracy and precision of the experiment ( ).

In discuss talk about the incubation temp has no effect

## Methods

Please refer to the methodology section of the practical schedule. Lab coats and gloves were worn for safety. There were no changes made to the protocol. However, the incubation time was increased and gels were not transferred to a humid box which contained damp filter paper and glass rod supports before filling the wells.

## Results:

## Standard C3 concentration (µg/ ml)

Graph 1 shows a scatter diagram of precipitate diameter against the standard C3 concentration. The line shows linear regression (y = 0. 0146x + 7. 3913) and the correlation coefficient of the data was plotted (r = 0. 99).

## Log C3 concentration (µg/ ml)

Graph 2 shows a scatter diagram of precipitate diameter (mm2) against log C3 concentration in. The line shows linear regression (y = 206. 67x – 340. 53) and the correlation coefficient calculated was r = 0. 96.

The correlation coefficient value was obtained from the calibration curves (graph 1 and graph 2). There is a close correlation between the values of the standards which is confirmed by the correlation coefficient (R2) obtained form the two graphs. Precipitate diameter against standard concentration of C3 was plotted and the correlation coefficient value obtained was 0. 99. The square diameter of the precipitate was plotted against the log concentration of C3 complement in graph 2 and the correlation coefficient value obtained was 0. 96. The difference between the two correlation coefficient values is 0. 03 which indicates that there is no significant difference between the two graphs and the values of both the graphs are précis. However, the correlation coefficient value of graph 1 is higher and it is more precise than graph 2.

## Calculating percentage error of reference:

From graph 1:

percentage error = value found – reference value x 100

reference value

internal reference = 400ï€ ï­g/ ml C3

Percentage error = I 247. 2 – 400 I x 100 = 38. 2 %

400

From graph 2:

Percentage error = I 171. 07 – 400 I x 100 = 57. 2%

400

The known reference concentration value provided for this experiment was 400μg/ml which was placed in the gel. Using both the graphs the precipitate was measured and used to determine the concentration of the reference precipitate. Using the value obtained the percentage error was calculated. The percentage error calculated from graph one is 38. 2% and from graph two is 57. 2% (which is higher than graph 1). The value obtained from graph two is 19 % higher than the value obtained from graph one. This suggests that the reference value obtained from graph 1 has lower percentage error compared to graph 2 and is more accurate. However, both the reference values are lower than the known reference value. This suggests that the single radial immunodiffusion technique is not an accurate method to determine C3 concentration in a patient’s serum.

The concentration of C3 in the reference serum sample calculated using graph one is 247. 2 µg/ ml and graph two is 171. 07 µg/ ml. The difference between the reference serum values is 76. 13 µg/ ml. The test serum value measured from graph 1 is 178. 7 µg/ ml and graph 2 is 135. 38 µg/ ml. The difference between the test serum sample values is 43. 22 µg/ ml. The test serum values obtained from the two graphs show that the patient has a lower concentration of C3 complement (as the values are lower than the normal reference range).

## Discussion:

The method used in this experiment (single radial immunodiffusion) is a simple technique used to measure specific serum proteins. The advantage of single radial immunodiffusion assays is that it can measure immunoglobulin and complement in serum as well as it can distinguish between the different classes of the antibody. The advantage of this technique is that it provides physical and later serological data which is of great importance to clinicians ( ). One of the requirements of the method is polyclonal antibody. The method does not require monoclonal antibody as the complex formation occurs on more than one binding site of the antigen.

In this experiment precipitate rings were formed on the gel by the standards, test and reference serum samples (as seen in figure 1) and their diameters were measured which are dependent on the C3 concentration. The single radial immunodiffusion plates were prepared with agarose gel which contained antibodies to the antigens. The wells in the agarose were filled with the samples (standard 1 to 4, test serum and reference serum samples) and were allowed to diffuse for 48 hours (the incubation time in this experiment was increased). The 6 samples were added in duplicates to 12 wells of the agarose gel. The results show that as the concentration of the standards increases (75 µg/ml to 600 µg/ml) the size of the diameter increases (7 mm to 15 mm). This might be due to that fact that as the concentration of antiserum increases more antigen- antibody complexes are formed that precipitate to form a disc around the well. As the concentration of antigen increases the size of the precipitin increases until a maximal precipitin is formed. However, if the concentration of antigen is in excess the size of the precipitin decreases. A precipitin curve when plotted consists of three regions (zones of the curve). The early part of the curve shows the antibody excess and the later shows the antigen excess where the diameter of the precipitant is small. In between the two zones is a zone of equivalence where precipitation is at its maximum level ( ).

In this experiment, the concentration of C3 in the test serum sample was measured and the accuracy and precision of single radial immunodiffusion was estimated. Due to inadequate staining of the gel, another gel (figure 1) was provided for this report. The precipitate diameters of the samples in each well were measured from the gel provided (figure 1). The correlation coefficient was measured from the two graphs which were used to assess the precision of the method. Correlation coefficient value indicates how strong the relationship of the precipitate and concentration of C3 complement is. A correlation coefficient value (closer to one) indicates that there is a good relationship between the variables and that the test is precise. There is no significant difference between the correlation coefficient values obtained from graph one (0. 99) and two (0. 96). However, the value of graph one is slightly higher than graph 2 (19 %) which suggests that correlation between the variables in graph one is very strong and the values are more precise than in graph two. The values of the diameters of the precipitate in duplicates (table 1) were close to each other which agree with the suggestion that the values are precise. Therefore, this can suggest that there was no error in pipetting the samples into the wells of the gel.

The percentage error calculated in this experiment is measured by measuring the concentration of unknown reference serum samples from graph one and graph two. By comparing the values to each other and choosing the reference value from the graph that had lower percentage error and high correlation coefficient showed accuracy of the method shows the accuracy of the method used in this exeperiment.. Percentage error is a statistical test used to determine the accuracy of a test. A low percentage error value indicates that the experiment is more accurate. The percentage error of graph one was 38. 2 % and graph two was 57. 2 %. Graph one has a lower percentage value which suggests that graph one is more accurate than graph two. The unknown reference values were compared to the reference value provided in this experiment (400μg/ml). The values were found to be 247. 2 μg/ml (graph 1) and 171. 07 μg/ml (graph 2) which are lower than the normal reference range 400μg/ml. This shows that the method of single radial immunodiffusion is not an accurate method for determining the concentration of complement C3. As the difference between the value of 247. 2 μg/ml (from graph one) and known reference range is 152. 8 μg/ml, which is quite a big difference. The accuracy and precision of a calibration curve is essential in a clinical setting to determine the concentration of C3 complement in a patient’s serum. Complement testing is usually done when a person has symptoms of an autoimmune disorder such as Systemic lupus Erythematosus. The method used in this experiment can be used to check patient’s complement system.

Both C3 and C4 complements are tested to determine whether there is deficiency in the complement system that is causing harm to a patient’s disease or condition. Complement testing is important as it helps in the diagnoses of a patient and it identifies the cause of microbial infections (angioedema or inflammation). It may also be monitored with immune complex diseases such as glomerulonephritis and vasculitis. During the formation of immune complexes, the complements clear them from the blood and their level decreases.

The levels of C3 and C4 are often measured when deficiencies are suspected to give an idea of the severity of the condition which is linked to the decrease in the level of complement. The levels are decreased due to a deficiency or increased due to consumption. However, in acute or chronic condition the levels of the complements return to normal if the underling condition is resolved. The level of C3 in this experiment is lower than the reference value which suggests that the patient serum tested in this experiment is expected to have a problem, probably an illness (Parslow et al., 2001).

There are several problems in measuring C3 concentration which includes the array of reference ranges. Different studies stated different reference ranges to compare the concentration of C3 found in the test serum sample of males (88 to 252 mg/dL) and females 88 to 206 mg/dL (Borigin., 2009a). Borigin, 2009b stated a combine reference range of 75-135 mg/dl (Borigin 2009b). The different types of reference ranges indicated in various studies can fail to provide accurate information for the diagnosis of patients (Hussain et al., 2008). Another study quoted a range of 50-120 mg/dl, this variation in normal references for C3. The value is lower compared to all the ranges as well as the range provided during the experiment. The test serum sample has a concentration of 178. 7 μg/ml (from graph one), after converting to the sample units as the reference ranges, it is noted that this value is lower compared to all the ranges. The value suggests that the patient has an immune deficiency of C3 which can be suspected to be systemic lupus erythmatosis (North, 2010). This is a type of disorder that is associated with the low level of C3 concentration as well as a number of clinical manifestations. In a study, majority of the patients with a low C3 concentration were affected with systemic lupus erythmatosis (Hussain et al., 2008; Agarwal et al., 2009).

There might have been errors in this experiment such as damage to the gel might have occurred as the plates were stored for a long period of time. Gels that are uneven can result in inaccurate results. Inclusion of air bubbles in the wells can lead to inadequate filling of the wells. Therefore, extra care must be taken when filling the wells with samples to avoid spillage of the antigen outside the well. In addition, size of the well can also lead to inaccuracy of the test.

Conclusion: In conclusion, the difference in the accuracy of the two graphs shows that the test is inaccurate. Several studies disagree with this conclusion and their results have shown how reliable the technique of single radial immunodiffusion is in the determining C3 concentration ( ). The method is an important technique used in the clinical setting to measure C3 concentration in a patient’s serum which can indicate the presence of an immune complex disease. In this experiment the standard C3 concentration along with test and reference serum samples in duplicates were loaded into the gel. Graph one is more accurate and precise compared to graph two as it had the highest correlation coefficient value and lowest percentage error. The test serum values were measured from the two graphs and they were compared. The difference between the two test serum values (178. 7 μg/ml and 35. 38 μg/ml) was 43. 22 μg/ml. This can indicate that such a difference in the values can have great impact on the diagnosis of a patient. The patient had a lower C3 concentration and this is because the patient has immune complex disease for example systemic lupus erythmatosis. The concentration of C3 complement when compared to the normal reference range ( ) was low. The method of single radial immunodiffusion is not a good reliable method for diagnosis as the reference values obtained by the two graphs were not close to the reference range provided for the experiment 400µg/ ml and to the reference ranges used in other studies ( ).

In this experiment the human serum of unknown C3 concentration was loaded into the gel. Graph one was more precise and accurate, due to the highest correlation coefficient value and the lowest percentage error, therefore was used to determine the concentration of C3 in the test sample (247. 2μg/ ml). Although the concentration of C3 in the test sample was determined by both graphs, to demonstrate how two calibration curves plotted for the same test can produce huge differences in their results. The difference was 78μg/ml; such a variation has direct effect on the diagnosis a patient receives.

## Questions:

Q1. List three factors which will affect the diameter of the precipitate in the agarose.

One of the factors that can affect the diameter of the precipitate in the gel is the incubation time at which the precipitates are formed in the wells, as this allows the antibody and antigens to from complexes with each other. The diameter of the precipitate is directly proportional to the concentration of C3 complement. If the incubation time was decreased in this experiment then the size of the diameter produced might have been smaller as less time is allowed for the antigen- antibody complexes to form and to form a precipitate in the gel.

Several other factors can affect the diameter of the precipitate, such as environmental factors and compound concentrations. The concentration of antigen can affect the diameter of a precipitate (Bailey, 1996). The concentration of the antigens is proportional to the precipitate, as the concentration of the antigen increases the size of the precipitate formed around the wells is high. As the concentration of the external reactant (antibody) or the internal reactant (antigen) increases, the likely-hood of antigen- antibody complexes to form also increases. However, if the concentration of antibody is low then fewer antigen-antibody complexes would form which can result in a smaller diameter of precipitate. High antibody concentration is required to produce stainable and visible precipitin zone (wood et al., 1979). Similarly, different antibodies can precipitate the same antigen to a varying degree ().

However, the antibody would become saturated with the antigen and the number of immune complexes formed would equilibrate, as the antibody becomes the limiting factor, therefore the diameter of the precipitate would also reach a peak size. Similarly, the weaker the internal reactant the further the external reactant would diffuse before equilibrium is reached. (might produce greater diameter check)

The concentration of C3 present in the patient serum can affect the diameter of the precipitate formed by the antibody- antigen complexes as it diffuses radially during incubation time causing the complexes to form a precipitate in the agarose gel. If the concentration of C3 is low then the size of the diameter of the precipitate would be small. On the other hand if the concentration of the C3 is high then the size of the diameter would also be big.

The pattern of wells cut into the gel could also have an effect on the precipitate diameter. The molecular size of the antigen and antibody determines the rate of diffusion in the gel. Therefore, if the pore size in the gel is increased, the compound would diffuse more rapidly and the size of precipitate would also increase. However, if the pore sizes are too big then this can increase the possibility of antigens and antibodies from being lost in the gel.

Q2. If the antiserum concentration is decreased, what is the resulting effect on the zone diameter?

When antigen is in excess the antibody and antigen complexes are more soluble in the gel. This is visible in figure 1, the outer edge of the precipitate is darker compared to the inner edge, as fewer antigens are present in the outer edge compared to the inner edge which is nearer to the well (Xing et al., 1998). In addition, the higher the concentration of antiserum, more antibody- antigen complexes will be formed which will result in high sensitivity. Therefore, if the antiserum concentration is decreased then fewer antibody- antigen complexes will be formed and the diameter of the precipitate would be small.

Xing et al., 1998 disagrees with the statement by Bailey (1996) and their experiment shows an inverse relationship between antiserum concentration and zone size. Therefore as the concentration increased the diameter decreased, therefore if the antiserum concentration is decreased the diameter will increase this occurs because antibody-antigen complexes more soluble when antigen is in excess. This explains the appearance of the gel, the precipitate in the centre of the circle is lighter compared to the outer edge (appendix one). The centre is more concentrated with antigen as it is near the well and the outer edge is less concentrated, this explained why

Antigen-antibody complexes are small and soluble when in antigen excess. Therefore, precipitation near the center of the circle is usually less dense than it is near the circle’s outer edge, where antigen is less concentrated.

Q3. Will related complement components, for example C4, interfere with the assay? If not explain why?

The related complement, for example C4 will interfere with the assay as the fragments C4a (produced by the cleavage of C4) and C3a (produced by the cleavage of C3) are similar to each other, this suggests that there will be interference as they have similar binding (Michael, 2009). The anti- serum used in this practical and the anti- C3 antibody was specific to C3 complement which suggests that the binding sites are not identical to the C4 so there will not be any interference of C4.

Q4. Why can’t the above method be used to measure the serum IgE concentration? What method should be chosen to measure serum IgE and why?

Serum IgE can be measured by enzyme linked immunosorbent assay as it can measure molecules at lower concentrations. Whereas, single radial immunodiffusion cannot measure IgE as it is present in low concentrations in the body (Stites et al., 1999). Radio immunosorbent test (RIST) can be used to measure a wide range of IgE concentration and it is the most suitable and rapid method (Berrens and Bruynzeel., 2003).