Biochemical Techniques To Determine The Concentration Or Total Level Of Protein In A Solution

Introduction-

There are many biochemical techniques that can be used to determine the concentration or total level of protein in a solution, in a precise and accurate way. One method of quantifying protein from a sample is using protein assays (Illingworth, 2019). There are different protein assays such as the Lowry assay and Bradford assay (Answers, 2019). The assay used to measure the level of protein depends on several factors such as compatibility with the sample type and components, availability of spectrophotometer needed to measure the colour produced (absorbance) by the assay etc (Thermo Fisher Scientific, 2015) .

Protein quantification in patient urine samples is an important diagnostic tool because it is used to diagnose a disease or check for health issues (National Kidney Foundation, 2019). Healthy kidneys should only filter trace amounts into the urine because majority of protein molecules are too big for the filters (glomeruli). It is not normal to lose protein in urine but when this occurs it is called ‘proteinuria’ (Illingworth, 2019). Several proteins are found in urine however the most relevant to kidney disease is albumin and so it is the most common protein measured (NHS, 2019). Protein may appear in urine before the patient knows they have a problem. If there is protein in urine, then this is an indication of a kidney-related disease (National Kidney Foundation, 2019). Protein in urine is not normally obvious although it can be tested using a dip-stick test or by more sensitive lab tests such as using protein assays. The presence of protein in urine can be a warning sign that the kidneys are not functioning correctly (Illingworth, 2019). Examining protein levels in patient samples is important because it helps diagnose disease in the early stages and treat it before the problem progresses and becomes worse.

Various proteins make up the blood plasma and they help in carrying out different functions. This involves regulating volume of blood plasma as well as protein molecules which aid in transporting substances such as lipids, hormones etc (Hamlett, 2019). Albumin is the most abundant protein which can be measured in the plasma (Illingworth, 2019) . A healthy person should not have protein in their urine, with an albumin concentration of 20-30ug/ml or below being classed as normal. If albumin protein levels are higher than 30ug/ml, this can show the person may no longer be healthy. (Illingworth, 2019).

Albumin protein concentrations of 250ug/ml are classed as high and may be an indication of kidney damage also known as micro-albuminuria or proteinuria. Increased albumin concentration can suggest a variety of health problems such as bacterial infections of the urinary tract and/ or kidneys, eclampsia, diabetes and damage to the kidneys (Illingworth, 2019). To check for abnormal levels of albumin in urine, a precise screening test is required. This is an important diagnostic tool for recognising a developing kidney disease in its early stages. This test manages and decreases the increasing symptoms of chronic kidney disease (Toussaint, 2012). A patient that shows symptoms of chronic kidney disease will undergo a proteinuria uranalysis which screens for protein in the urine and then an overall evaluation of the urine sample is carried out, this checks for various substances that could be present in the urine including protein (AACC, 2016). For example, if a patient has diabetes, a direct indicator that

diabetes will evolve into diabetic nephropathy, is if there is an increase in albumin in urine (Tidy, 2017). Patients that are not diabetic and start to possess a protein concentration of above 30ug/ml are still in danger of developing chronic kidney disease. (Tidy, 2017).

The aim of the laboratory experiment was to use a Bradford assay to determine the concentration of albumin in 5 patient urine samples with high and normal control samples (Illingworth, 2019). To achieve the aim, the first step was to create an accurate calibration graph. Then use the calibration graph to determine the concentration of protein in each sample, finishing with a diagnosis of the patient samples. This was done by comparing them to the control samples to see if any patients have abnormal protein levels in the serum.

Methodology -

Implementing the Bradford assay method, eight standards of albumin concentrations are prepared. The protein concentrations in each standard are from 0ul, 25ul, 50ul, 75ul, 100ul, 250ul,500ul and 1000ul. Water is added to labelled microtubules with Bovine Serum Albumin 1mg/ml (1ug/ul). Duplicates of 100ul of each standard are added to corresponding labelled test tubes with 5ml of Bradford reagent and incubated at room temperature for 10 minutes, prior to being recorded at a wavelength of 595nm in the spectrometer. Absorbance values produced by the spectrometer of all standards and duplicates are recorded in excel and averaged. A calibration graph is generated from these averages, giving a y value and R2 value. The calibration graph calculates the y value in y=mx and is used to determine protein concentrations of every sample.

Patient samples are then used to calculate the amount of albumin in them. Duplicated samples (with 100ul of each patient sample as well as controls+5ml of Bradford reagent) are left to sit for 10 minutes at room temperature, then read at 595nm in spectrometer, giving the m value. These values are added to excel, and averaged. With these averages and x=m/y equation, a column bar chart is produced displaying the quantification of albumin concentration +/- standard deviation of patient samples.

Results-

The Albumin protein in urine is a crucial indicator for the diagnosis and prediction of diseases. Urine tests that have high levels of albumin are normally the first stages in recognising that there is a health issue. Specific symptoms which a patient shows may be an apparent sign that albumin levels are high.

Examining albumin levels will therefore substantially aid in determining disease. For this reason, it is most important to quantify albumin protein concentrations in patient samples to help in reducing fatality rates worldwide.

In this report, five patient samples were examined for albumin against a normal and a high controlled sample allowing for a clear comparison to be made. On obtaining the results displayed in figure one and two, the health issue can be identified, and a diagnosis can be established.

As displayed in figure 1, there is a linear relationship because when the concentration of

albumin doubles so does absorbance at a wavelength 595nm. Due to experimental error, figure 1 is not linear at higher concentrations of albumin. To fix this, the experiment needs to be repeated with a lower amount of protein in the standards. Furthermore, the R2 trendline must have a value of >0.95 to show a good fit. The R2 value (fig.1) is 0.1888 which is less than 0.95 and does not indicate a good fit. This is because of variation. The points on the graph in figure 1 are dispersed away from the trendline and the R2 value is a representation of the clustering of the data points around the trendline. The R2 value produced from this graph (fig.1) was due to human error and the experiment should be repeated.

The quantification of albumin concentrations for the 5 patient samples displays a range of concentrations (Fig. 2), with the lowest albumin concentration being patient five with a concentration of 35ug/ml whilst patient four had the highest albumin concentration of 142ug/ml.

Together, patient three and patient five have albumin concentrations (49ug/ml and 35ug/ml respectively) just below the normal control (59ug/ml). Whereas Patient two has an albumin concentration that is higher than the normal control, 77ug/ml compared to 35ug/ml, but there is not a significant difference as shown by the overlapping standard deviation error bars.

Though, patient one has a protein concentration that is significantly higher than the normal control (93ug/ml). In addition, patient four has an albumin concentration (142ug/ml) which is directly comparable to the abnormally high albumin level as shown by the high control (albumin concentration of 157ug/ml).

Discussion

In a healthy urine sample, normal protein albumin levels are within 20ug/ml and 30ug/ml, however, due to experimental error the normal control value in figure 2 is slightly higher (59ug/ml). Patient samples three and five (49ug/ml and 35ug/ml respectively) compared to the normal control (fig.2) have an albumin protein concentration of below normal (59ug/ml) suggesting they are healthy. Patient samples 1,2 and 4 have albumin concentrations higher than the normal control, however, only patient 4 has a high albumin concentration of 142ug/ml directly comparable to the high control. Protein concentrations from 30ug/ml to 250ug/ml are deemed as micro-albuminuria (Illingworth, 2019).

Patient 1- 35yr Female. The albumin concentration is 93ug/ml which is an abnormal level of protein and is significantly higher than the normal control (59ug/ml). Her symptoms suggest that she has preeclampsia as she is 30 weeks pregnant and has abnormal levels of protein (Beth, 2005). Headaches are a clear indication that the female is showing symptoms of preeclampsia (Gaither, 2018).

Patient 2 – 50yr Male. The albumin concentration is 77ug/ml, this is classified as micro-albuminuria as It is higher than the normal control (59ug/ml). The symptoms this male shows, being overweight, sharp pain in side of back and groin and blood in urine suggest that he has a large kidney stone (NHS, 2019)

Patient 3- 40yr Female. The albumin concentration is 49ug/ml which is just below the normal control of 59ug/ml. This shows that this female has a small amount of protein in her urine and can be classed as healthy.

Patient 4 -23yr Female. The albumin protein concentration is 142ug/ml which is significantly higher than the normal control (59ug/ml) and is directly comparable to the high control (157ug/ml). This patient has frequent urination, pain whilst passing, urge to urinate, urine odour and back pain. These symptoms suggest that the patient may be suffering from a Urinary Tract Infection as they are more common in women than men (Watson, 2016).

Patient 5 – 70yr Male. The albumin protein concentration is 35ug/ml which is less than the normal control of 59ug/ml. This indicates this male has a very small amount of protein in their urine and can be classed as healthy. However, the symptoms that the patient is showing suggest that he may still be recovering from a flu he had two weeks ago. The patient's immune system is weakened because of old age and may take more time in recovering.

References-

1. AACC. (2016). Lab tests online, protein in urine (proteinuria), 21st April, p.g. 2
2. Answers (2019) what is protein assay?.[online].Available at: https://www.answers.com/Q/What_is_protein_assay (Accessed: 18th April 2019)
3. Beth Israel Deaconess Medical Centre. (2005) Protein in urine may warn of preecalmpsia risk in pregnant women, science daily.[online]. Available at: https://www.sciencedaily.com/releases/2005/01/050111122343.htm (Accessed: 20th April 2019)
4. Gaither,k. (2018). Preeclampsia and Ecmlapsia, WebMD.[online]. Available at: https://www.webmd.com/baby/guide/preeclampsia-eclampsia#3 (Accessed: 20th April 2019)
5. Hamlett, S. (2019) what proteins are in blood plasma, LIVESTRONG, [online]. Available at: https://www.livestrong.com/article/190457-what-proteins-are-in-blood-plasma/ (Accessed: 19th April 2019)
6. Illingworth, T. (2019) Protein determination laboratory, PowerPoint from protein determination lab experiment, 4by514 practical methods in medical biosciences. University of Derby, unpublished.
7. Illingworth, T. (2019) Protein Determination laboratory Schedule, lab manual for protein determination experiment, 4by514 practical methods in medical biosciences. University of Derby, unpublished.
8. National Kidney Foundation (2019) why are patients asked for urine samples?. [online]. Available at: https://www.kidney.org/news/kidneyCare/spring10/UrineSamples (Accessed: 18th April 2019)
9. NHS. (2019) Protein in the urine (proteinuria), kidney research uk pdf.[online] Available at: https://www.nhs.uk/Livewell/Kidneyhealth/Documents/protein%20in%20urine.pdf (Accessed: 20th April 2019)
10. NHS. (2019). Kidney stone symptoms, NHS.[online]. Available at: https://www.nhs.uk/conditions/kidney-stones/symptoms/# (Accessed:20th April 2019)
11. Thermo Fisher Scientific. (2015). Overview of protein assays. [online]. Available at: www.thermofisher.com/uk/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-protein-assays.html (Accessed: 19th April 2019)
12. Tidy, C. (2017)Diabetic kidney disease, patient.info.[online]Availble at: https://patient.info/diabetes/diabetes-mellitus-leaflet/diabetic-kidney-disease (Accessed: 20th April 2019)

13. Toussaint, N. (2012) screening for early chronic kidney disease, CARI [online]. Available at: http://www.cari.org.au/CKD/CKD%20early/Screening_CKD.pdf (Accessed: 19th April 2019)
14. Watson, M.J. (2016). What to know about protein in the urine (proteinuria), doctors health press.[online]. Available at: https://www.doctorshealthpress.com/general-health-articles/what-to-know-about-protein-in-urine-proteinuria/ (Accessed: 20th April 2019)