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Determination of the Amount of Bilirubin content in Urine with the help of Spectrophotometry method by using boiled rice

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Abstract

Bilirubin is a compound which is yellow in colour, occurs in the normal catabolic pathway hich is the breakdown product of heme in vertebrates. This catabolism is a necessary process in the body's clearance of waste products that arise from the destruction of aged red blood cells. Bilirubin is of two types 1.Conjugated (direct) Bilirubin 2.Unconjugated (indirect) Bilirubin. The un-conjugated Bilirubin is Albumin-bound in serum and never present in urine whereas Conjugated Bilirubin is unbounded in serum and it is present in urine. Our proposed idea is about the determination of amount of Conjugated Bilirubin with the help of boiled rice grains using spectrophotometer instead of blood test. We propose this technique because the Boiled Rice grains have the capacity to absorb Bilirubin pigment. Spectrophotometer is used to detect the amount of Bilirubin absorbed in the rice

INTRODUCTION:

Bilirubin occurs in the normal catabolic pathway of all vertebrates. This catabolism is Urine bilirubin may also be clinically significant. Bilirubin is not normally detectable in the urine of healthy people. If the amount of conjugated bilirubin in blood becomes elevated, e.g. due to liver disease, high amount of conjugated bilirubin.a necessary process in the body's clearance of waste products that arise from the destruction of aged red blood cells. Bilirubin present in high level may cause certain discharged in the urine, indicating a pathological process. Un conjugated bilirubin is not water-soluble and so is not excreted in the urine. Testing urine for both bilirubin diseases which is responsible for the yellow colour of lesion, marks in various parts in our body and the yellow discoloration in urine. Its subsequent breakdown products, such as stercobilin, cause the brown color and urobilinogen can

help differentiate Obstructive liver disease from other causes of jaundice. The detection of direct (conjugated) bilirubin in urine is identified with the help of boiledricegrains. of feces. Urobilin

is the main component which causes straw-yellow color in urine. An increased amount of bilirubin leads to jaundice. Traditional techniques employed the usage of boiled rice grains which has the capability to absorb bilirubin. The boiled rice grains absorbed bilirubin pigment and changes from their normal colour to yellow colour. The intensity of the colour changes depends on the amount of bilirubin pigment present in urine. By the help of spectrophotometer it is easy to find the amount of absorbed bilirubin in rice grains.

The selection of rice grains plays a major role in this technique in which different varieties of rice have different absorption capacity. We select the hybrid variety rice named as "Taichung65 and Myang Ebos 6080/2 "which is commonly known as 'Ponni'.

2.1 Materials

Urine sample, Boiled rice, Spectrophotometer, Containers, Test tube

2.2 Method

In this method we make use normal and abnormal persons. First of all we want to collect the fresh urine sample from that persons. Some prescription drugs can cause a false positive, or a higher-than-normal reading of bilirubin in your body. Some false positives results indicates high bilirubin level that aren"t necessarily associated with liver disease. If the person takes following drug regularly are allowed to stop for two days before the testing of bilirubin in urine.: The following drugs are:

- 1. diuretics
- 2. birth control pills
- 3. steroids
- 4. barbiturates
- 5. sulfonamides

Urine analysis is painless, but it requires a time commitment and attention to detail. A thorough and accurate test requires to collect urine. The urine sample collected in morning is highly concentrated. Collect the samples from all the persons in the containers. After the collection of urineSamples, the cup of rice mentioned above is boiled in distilled water. First inspect the urine sample in the spectrophotometer and measure absorbance at 490nm wavelength.

Then pour some rice grains in the urine sample and left it undisturbed for regular interval of time. Filter out the grains from the urine and measure the absorbance at same wavelength once again on that tested grains. Now take the necessary calculation to get the result.

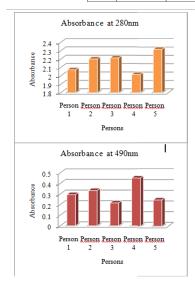
Two parameters have been made into considerations, they are absorbance and concentrations. i.e Absorbance α Concentration

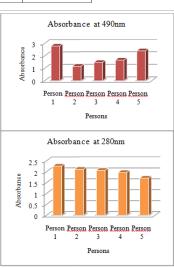
3. Selection of Wavelength

Wavelength at which the samples are inspected want to be identified. The normal samples and abnormal samples inspected at different wavelength are tabulated below:

Table 3.1: Normal urine sample

			_
S.no	Urine Samples	Absorbance at 280nm	Absorbance at 490nm
1	Person 1	2.072	0.293
2	Person 2	2.201	0.332
3	Person 3	2.214	0.215
4	Person 4	2.016	0.451
5	Person 5	2.318	0.246

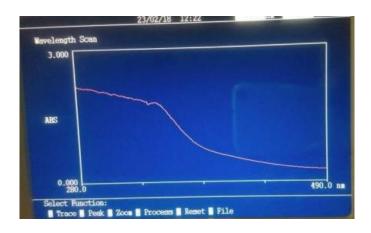




Jaundice infected urine sample

S.n	Urine	Absorbance	Absorbance	
O	Samples	at 280nm	at 490nm	
1	Person 1	2.259	2.780	
2	Person 2	2.117	1.157	
3	Person 3	2.072	1.459	
4	Person 4	1.972	1.646	
-	Ferson 4	1.972	1.040	
5	Person 5	1.714	2.385	

From the table it is identified that there is lot of deviation obtained between normal and Bilirubin infected urine sample at 490nm Which also have been proven by following graph:



Graph plot between 280nm and 490nm in normal urine sample



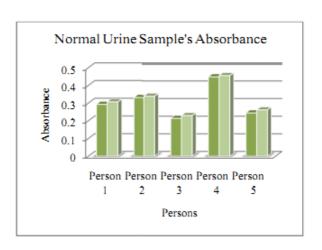
Graph plots between 280nm and 490nm in Bilirubin infected urine sample

4. Calculation

Values of Absorbance of Urine samples in tested rice grains at 490nm Normal urine sample

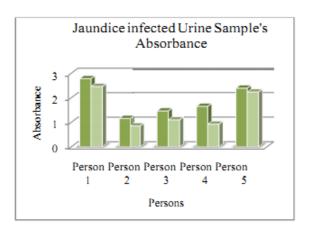
Normal urine sample

S.no	Urine Samples	Absorbanc e before adding rice grains	Absorbanc e after adding rice grains
1	Person 1	0.293	0.307
2	Person 2	0.332	0.341
3	Person 3	0.215	0.231
4	Person 4	0.451	0.458
5	Person 5	0.246	0.263



Jaundice infected urine sample

S.no	Urine	Absorbance	Absorbanc
	Samples		e after
		adding rice	adding rice
		grains	grains
1	Person 1	2.780	2.461
2	Person 2	1.157	0.847
3	Person 3	1.459	1.097
4	Person 4	1.646	0.921
5	Person 5	2.385	2.229



Amount of Absorbance of bilirubin content in urine = Absorbance obtained before adding rice grains - Absorbance obtained after removal of rice grains

S.no	Urine	Amount o	f
	Samples	Bilirubin	
		Absorbance	
1	Person 1	0.319	
2	Person 2	0.310	
3	Person 3	0.362	
4	Person 4	0.725	
5	Person 5	0.156	

Calculation of Concentration Concentration = (Absorbance - 0.080) /0.440

Person 1:

Concentration = (Absorbance - 0.080) / 0.440 = (0.319 - 0.080) / 0.440 =0.543 Moles / litre

Person 2:

Concentration = (Absorbance - 0.080) / 0.440 = (0.310 - 0.080) / 0.440 = 0.522

```
Moles/litre
Person 3:
Concentration = (Absorbance - 0.080) / 0.440 = (0.362 - 0.080) / 0.440 = 0.640
Moles/litre
Person 4:
Concentration = (Absorbance - 0.080) /
0.440 = (0.725 - 0.080) / 0.440 = 1.465
moles/litre
Person 5:
Concentration = (Absorbance - 0.080) / 0.440
= (0.156 - 0.080) / 0.440 = 0.172 moles/litre
```

6. RESULT

Concentration and Absorbance

S.no	Urine	Amount of	Amount
	Samples	bilirubin	of
			Bilirubin
		absorbance	Concentr
			ation
			(moles/lit
			re)
1	Person 1	0.319	0.543
2	Person 2	0.310	0.522
3	Person 3	0.362	0.640
4	Person 4	0.725	1.465
5	Person 5	0.156	0.172

7. INFERENCE

In phototherapy technique light beam is used a major source. These light waves are absorbed by your baby's skin and blood and change bilirubin into products, which can pass through their system. The term "bili" in all these names refers to bile are derived from the haem by an oxidative reaction catalyzed by haem oxygenase. Chlorophyll too is derived from haem (Heme). Chlorophyll is vital for photosynthesis, which allows plants to absorb energy from light which is a haem product. Meanwhile the bile pigment bilirubin a haem product whose structure is also affected by light exposure, a fact exploited in the phototherapy of jaundiced newborns. From that we can find out that there is a relation between light and bilirubin which has been employed in the above technique.

7.References

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