

Concentrations of HCG Hormone in Urine by Optical means

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Abstract:

Human chorionic Gonadotropin (HCG) in the urine of pregnant women, this in addition to the standard samples. Fluorescence tests results showed good compatibility with those of the radioimmunity assay tests, as a prominent peak appeared in the curve of the fluorescence intensity at the wavelength 475 nanometer in the urine samples during pregnancy.

المستخلص:

أجريت الفحوصات على هرمون HCG في إدرار الحوامل، فضلاً عن النماذج القياسية وقد كشف فحص الفلورة على تطابق جيد مع نتائج الطريقة المناعية. إذ ظهرت قمة بارزة في منحنى شدة الفلورة عند الطول الموجي (475) نانومتر في أنموذج الإدرار خلال مدة الحمل.

Introduction:

Human chorionic gonadotropin (HCG) is so important to diagnosis the human pregnancy. HCG was found in urine of pregnancy.

Commercial HCG assays have usually not been validated for determination of HCG in urine. HCG can be detected in urine by highly specific mass spectrometric methods.

In men the concentrations are lower but mostly are measurable by highly sensitive assays. In those >50 , it is 2 IU/L (6.1pmol/L) ^[1]. Concentration as great as 3-4 IU/L are occasionally observes in healthy men.

In women with normal pregnancy the concentration of HCG in serum starts to increase 7-11 days after ovulation, corresponding to 21-25 days after the last menstrual period ^[2]. The increase is nearly exponential during the first 5 weeks after implantation ^[3]. As measured by our method, the concentration peak at $\sim 110,000$ IU/L, during week 8-10 after the last menstrual period (i.e. 5-7 weeks after implantation).

After which they decrease and reach a nadir of 36000 IU/L at the beginning of the second trimester ^[4]. These values are method depended ^[5, 6].

There is small increase before delivery ^[7], and the concentration of HCG and its subunits are high in multiple pregnancies than in singleton ones ^[8]. Individual variation in HCG concentration is large ^[4].

After normal delivery, the HCG concentrations decrease with a half time of about 24-32 hr ^[8] and normalize with 1-3 weeks ^[9,10]. Serum HCG concentration after first-trimester abortion may take 4-5 weeks to return to normal values ^[11]. The concentrations in urine are 4000- fold those in serum ^[12].

Theoretical Aspect:

Relation between Fluorescence Intensity and concentration

The basic equation defining the relationship of fluorescence to concentration is:

$$F = \phi I_0 (1 - e^{-\epsilon bc})$$

Where:

ϕ : the quantum efficiency,

I_0 : the incident radiant power,

ϵ : the molar absorptivity,

b : the length of the cell,

c : the molar concentration.

The basic fluorescence intensity-concentration equation indicates that there are three major factors other than concentration that affects the fluorescence intensity.

a- The quantum efficiency ϕ , the greater the value of ϕ , the greater will be the fluorescence.

b- The intensity of incident radiation I_0 .

c- The molar absorplivity

For every dilute solution the equation reduces to one comparable to Beer's law in spectrophotometry:

$$F = k \phi I_0 \epsilon b c$$

The relation between the fluorescence and the concentration should be linear at low concentration. At high concentration quenching becomes so great that the fluorescence intensity decreases.

Review of the previous Experimental works:

At (1928) A Shime and Zottech were found in the gonadotropin in urine of pregnancy. Disclosed the gonadortopin hormone is so important to diagnosis the human pregnancy. Figure (1) explains the quantity of HCG in urine ^[13]. Figure (2) explain the HCG in serum ^[13]. A Sheim and Zottech were measured by biological method. In (1965) immunological tests were used.

Serum assays: Specific determination of HCG in serum from non pregnant subjects was first performed by RIA with antiserum SBb ^[14].

Urine assays:

Because of the large differences in assay design and specificity for different forms of HCG, the concentrations measured are highly dependent on assay used ^[5,6]. The concentrations measured in quality control samples may over five-fold ^[5]. The relative amounts of various HCG forms also depend on the clinical situation of the subject tested. All these factors contribute to the large variation in reported HCG concentrations.

Sample Preparation:

Urine samples for estimation of HCG are collected and tested immediately. Initially, urine hormone estimations were performed using fluorescence methods. The result summarized below of our scheme corresponding values for the Kit methods reported by the manufacturers compare favorably with our own, thereby giving reasonable assurance.

Results & Discussion:

a- HCG

The fluorescence wavelength of solution containing HCG is found at 470 nm.

b- Appearance of the fluorescence spectrum of HCG

The fluorescence spectrum were performed on urine samples of pregnant women and determine the relative intensity of the fluorescence spectrum which was directly related with concentrations of the HCG that was increasing during pregnancy.

c- The relative fluorescence intensity less than 20% indicates (Negative) pregnancy. As shown in figure (3).

The relative fluorescence intensity more than 100% indicates (positive) pregnancy as shown in figure (4).

At parameters

Abscissa scale X₂: Ordinate scale X₂

Scan speed very fast: Sensitivity high

Excitation slit 10nm: Emission slit 10nm

Excitation wavelength 475nm: Emission wavelength 475nm.

Conclusion:

1- The HCG Hormone of urine samples were all detected by fluorescence technique where the wavelength of excitation and emission as stated due to the peak of corresponding hormone. Which states an exact and genuine identification as compares to authentic RIA identification.

2- <u>Hormone</u>	<u>$\lambda_{\text{exi}}(\text{nm})$</u>	<u>$\lambda_{\text{emi}}(\text{nm})$</u>
HCG	475	475

3- The fluorescence spectrum shows the area under the fluorescence curve represent the quantitative assessment of that particular hormone assay in human body.

4- Urine concentrations of Gonadotropin (HCG) were measured, where, in practice the concentration of impurities in urine should be several times less than this limits in serum.

5- The hormone HCG has a resonance fluorescence spectrum (absorption energy = emission energy) obviously, when excitation occurs the states transfer from the ground level to the highest excitation level, these levels are populated (low density) and this leads to decrease of possibility of collision or completely disappeared, finally the quenching become less.

References:

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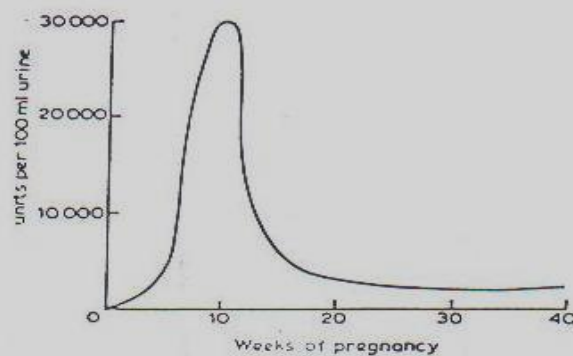


Figure (1) Concentration of HCG in urine during pregnancy

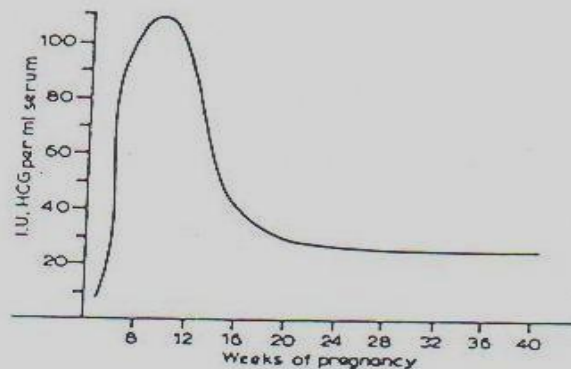
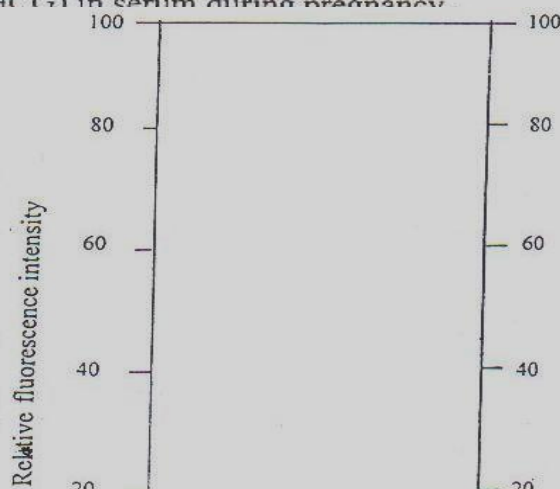


Figure (2) Concentration of chorionic gonadotrophin (HCG) in serum during pregnancy



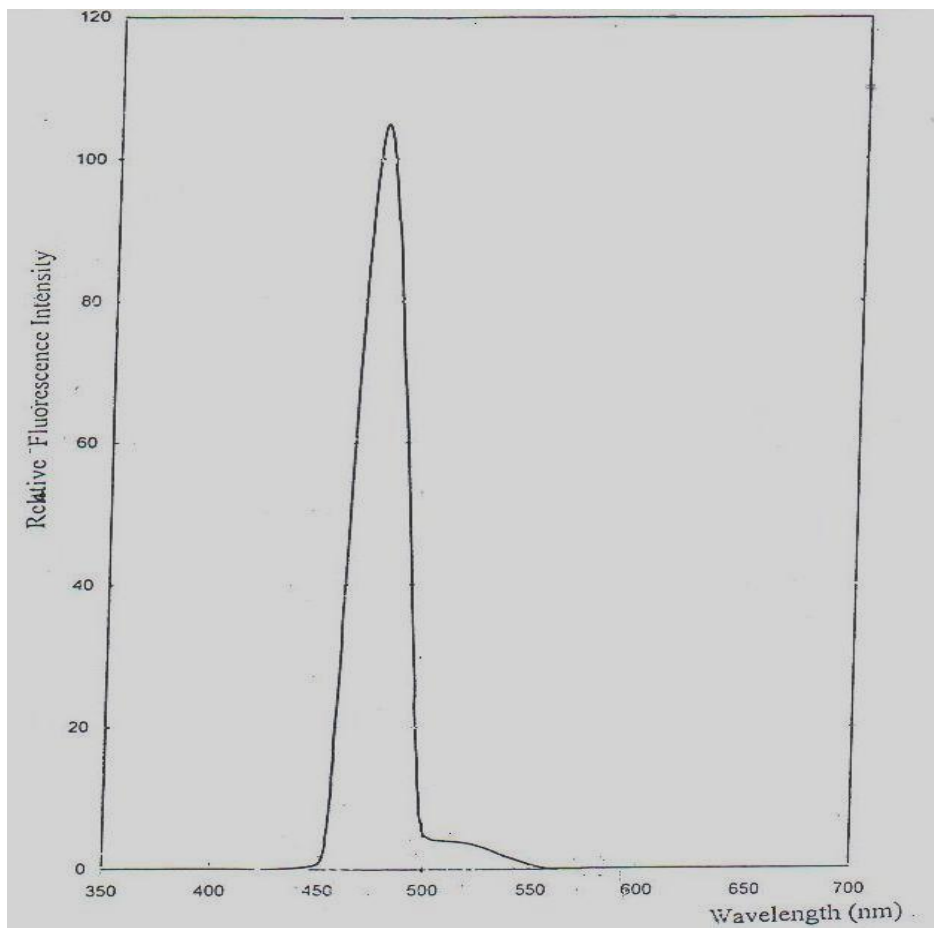


Figure (4) illustrates the fluorescence spectrum for (+) pregnancy.
The relative fluorescence intensity more than 100%