

**A SENSITIVE IMMUNOASSAY FOR HUMAN  
GROWTH HORMONE (hGH) IN URINE USING  
ALKALINE PHOSPHATASE LABELED ANTIBODY  
AND CHEMILUMINESCENT SUBSTRATE**

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**ABSTRACT**

*The assay of human Growth hormone (hGH) in urine offer potential advantages in serum. The measurement of hGH in urine remains an analytical challenge as concentrations are low and many samples assay at the limit of detection of the assay used. From the analysis of the assay kinetic parameters of a two- site immunoradiometric assay for hGH, we predicted that residual signal was available at analyte concentration below those detectable with I125. We therefore sought to increase the sensitivity of the assay system by an alternative label. The assay was modified to make use of alkaline phosphatase as label instead of I125; we chose to detect the alkaline phosphatase label using as substrate the chemiluminescent substrate adamantly,4-methoxy -4- (3-phosphatephenyl) - Spiro (1,2-dioxetane-3,2`-adamantine) designates as PPD (Diagnostic Products). The assay was shown to be linear from 0.1 to 100 pg growth hormone per tube. This is equivalent to an hGH concentration of 0.05 to 50 pg / ml in a 2 ml of urine sample. Each standard was assayed 10 times to generate an Ekins plot of hGH concentration against standard deviation (Figure 3). Extrapolation to zero concentration gave a detection limit of 0.04 pg/ ml of growth hormone. Different urine samples (n= 17) gave recoveries of 93.9%± 4% at a growth hormone level of 12.5 pg / ml and 93%± 6%, (n= 10) at the level of 2.5 pg / ml. A total of 23 samples were analyzed by Netria hGH (IRMA) on dialysed urine and by the assay described. There was good agreement (r= 0.980) between both assays.*

## INTRODUCTION

The assay of human Growth hormone (hGH) in urine offer potential advantages in serum. The measurement of hGH in urine remains an analytical challenge as concentrations are low and many samples assay at the limit of detection of the assay used. Due to the low level of human hGH, only few assays, **Girard *et al.***,<sup>(3)</sup> **Hourd and Edwards**<sup>(1)</sup> and reviews **Hourd and Edwards**<sup>(2)</sup> are considered that the use of I125 as a label in sensitive two-sites labeled antibody assay constraints the potential sensitivity due to lack of signal.

From the analysis of the assay kinetic parameters of a two- site immunoradiometric assay for hGH, we predicted that residual signal was available at analyte concentration below those detectable with I125. We therefore sought to increase the sensitivity of the assay system by an alternative label. The assay was modified to make use of alkaline phosphatase as label instead of I125; we chose to detect the alkaline phosphatase label using as substrate the chemiluminescent substrate adamantly,4-methoxy-4-(3-phosphatephenyl)-Spiro(1,2-dioxetane-3,2'- adamantine) designates as PPD (Diagnostic Products).

## MATERIAL & METHODS

### ***Preparation of immunoglobulin fraction from polyclonal antiserum:***

A polyclonal anti hGH antibody was partly purified by mixing with 4 volumes of 0.1M acetic acid and adding caprylic acid slowly at the rate of 25 µl per ml of mixture. Precipitate was removed by centrifugation and the supernatant passed through 0.8 µm filter.

### ***Coating procedure:***

Polystyrene balls (6.4 mm diameter; from NBL Gene Science Lts) were washed, degassed and covered with 0.2 M phosphate buffer pH

7.2 before adding the antibody solution using 1 ml of serum per 5000 balls.

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After incubation for 3 hours at 37 °C, the coated balls were washed and stored in buffer until used.

### ***Preparation of labeled anti-hGH:***

The antibody conjugate was prepared by dialysis both alkaline phosphatase (Biozyme, calf intestine, 5 mg), and monoclonal antibody to hGH (10 mg) against 0.1 M Na<sub>2</sub>HPO<sub>4</sub> pH 6.8. These solutions were then mixed, 50µl of 1%glutaraldehyde (Sigma), added and incubated for 3 hours at room temperature. 0.1 M ethanolamine pH 7.0 was added, and the complex allowed standing for a further 2 hours before dialysis overnight against PBS pH 7.4.

### ***Assay procedure:***

Urine samples (2 ml) were incubated overnight with end-over end mixing at room temperature with antibody coated beads. The balls were washed thoroughly with Tween HEPES buffer pH 7.4. The balls were transferred to clean tubes, and 2 ml of HEPES buffer pH 7.4 (blocking agent contain 1 % BSA and 5% skimmed milk to reduce non-specific binding) and 100 µl labeled anti-hGH (200 ng) were added, followed by incubation overnight as before. After further washing, 200µl PPD substrate and 100 µl blocking agent were added. 2 hours incubation was found to be necessary to develop the maximum signal. Chem.-luminescence was measured in a Ciba-Corning Luminometer Model.

### ***Assay standardization:***

International Standard (80/505) was obtained from netria as standard for use in serum assay. These were diluted in horse serum to give rang of standards to give appropriate values, when 25 µl were diluted in 2 ml phosphate buffer which were then treated the same as urine samples to provide the dose response curve.

### ***Recovery experiments:***

Recoveries were estimated by mixing 2.5 pg (n= 10) or with 12.5 pg (n= 17) hGH standard with different urines.

## RESULTS

### ***Reduction of Non-specific Binding:***

Various combination of blocking agents was assessed in an attempt to minimize non-specific binding, BSA and skimmed milk together being the most effective. The inclusion of Tween in the wash buffer also helped in this respect. The order of addition of assay components (antibody- coated balls, labeled antibody, and sample) was also found to be important.

### ***Labeled antibody concentration:***

A low alkaline phosphatase labeled anti-hGH (200 ng) was optimized to reduce the assay blank, whilst maintaining signal.

### ***Standard curve:***

The assay was shown to be linear from 0.1 to 100 pg growth hormone per tube (**Figure 1**). This is equivalent to an hGH concentration of 0.05 to 50 pg / ml in a 2 ml of urine sample.

### ***Precision:***

The within batch CV% was 9% and the between batch CV% was 8% for levels of 1 pg growth hormone per 1 ml. the precision profile for growth hormone is shown in (**Figure 2**).

### ***Sensitivity of the assay:***

Each standard was assayed 10 times to generate an Ekins plot of hGH concentration against standard deviation (**Figure 3**). Extrapolation to zero concentration gave a detection limit of 0.04 pg/ml of growth hormone.

### ***Recovery:***

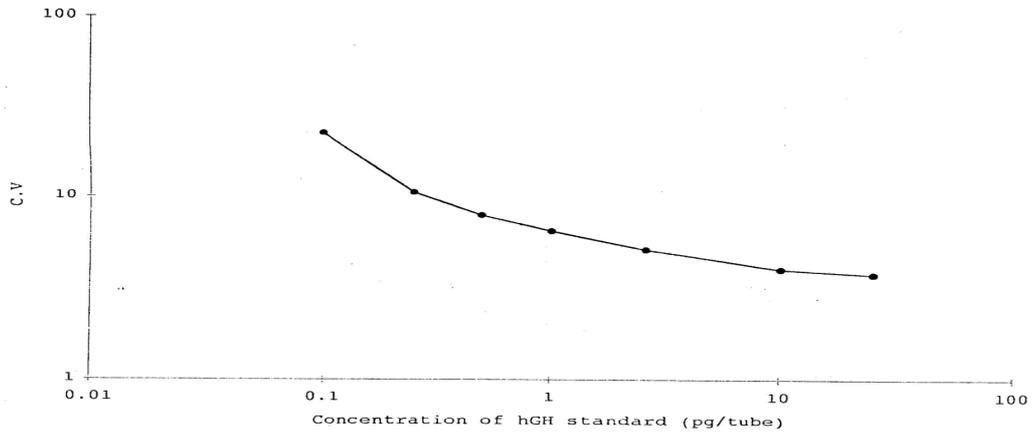
Different urine samples (n= 17) gave recoveries of 93.9%± 4% at a growth hormone level of 12.5 pg / ml and 93%± 6%, (n= 10) at the level of 2.5 pg / ml.

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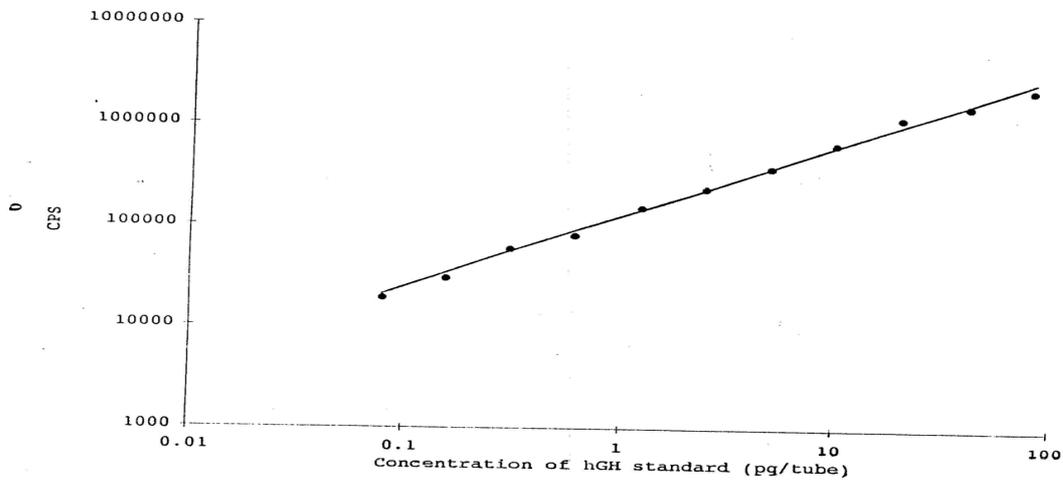
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### ***Comparison between immunoradiometric and chemiluminescent methods:***

A total of 23 samples were analyzed by Netria hGH (IRMA) on dialysed urine and by the assay described. There was good agreement ( $r= 0.980$ ) between both assays (**Figure 4**).



**Figure (1):** Measurements response plot of human growth hormone standard using PPD as substrate in chemiluminescence



**Figure (2) :** The Precision Profile of the Urinary Human Growth Hormon Using Chemiluminescence

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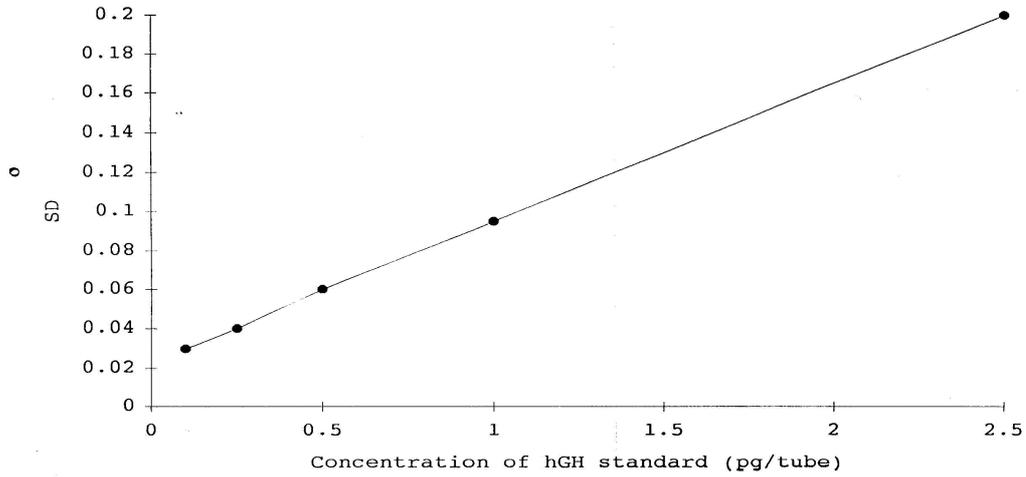


Figure (3): Ekins Plot to Determine Assay Sensitivity Derived from Precision Profile

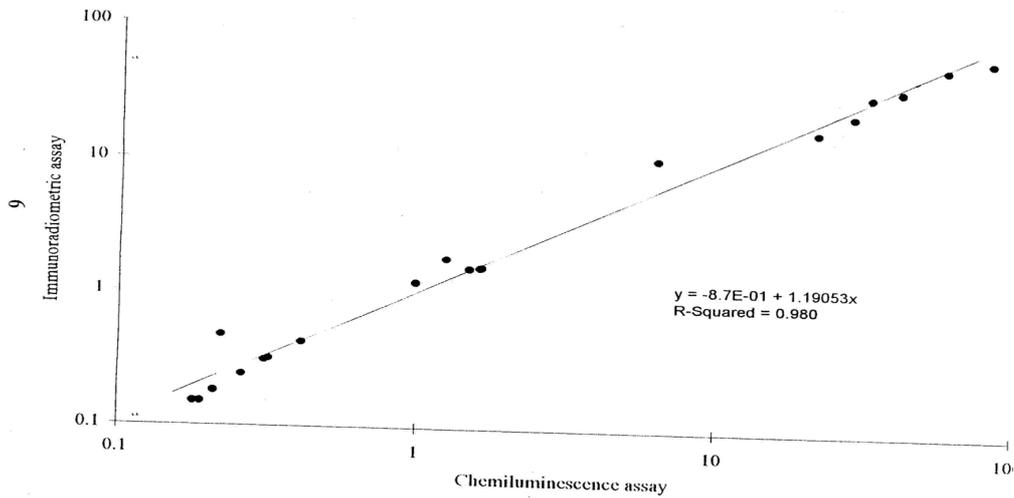


Figure (4): Comparison Study Between IRMA and Present Assay ( Log Plots )

## **DISCUSSION**

The assay uses a bead immunoextraction of urine with high affinity polyclonal antiserum. Reaction with the labeled antibody and signal development are subsequently allowed to take place in a controlled matrix. This configuration was chosen to reduce matrix interference which is a particular problem in urine which contains a range of solutes at varying concentrations. The excellent agreement with the reference method which uses prior dialysis supports this approach as does the recovery data in a wide range of different urine samples. In addition the immunoextraction permits a concentration of the hGH present which confers advantage in terms of sensitivity. In terms of precision the assay seems to be satisfactory in the clinical range especially at low levels.

## **REFERENCES**

- 1- **Hourd, P. and Edwards, R. (1989):** Measurement of human growth hormone in urine: Development and validation of a sensitivity and specific assay. *J Endocrinol*, 121: 167-175.
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- 3- **Girard, J., Erb, T., Pampalone, A., Eberle, A.N., and Baumann, J. B. (1988):** Growth hormone in urine: Development of an ultra-sensitive assay applicable to plasma and urine. *Horm Res.*, 28; 71-80.