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## Detection of Human Chorionic Gonadotropin in Urine by two Different Immunoassays.

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### Introduction

Human chorionic gonadotropin (hCG) may be abused by male athletes in sports. The International Olympic Committee has included it in the list of prohibited substances. The use of two different immunological methods is recommended to confirm the presence of hCG in urine.

In urine, hCG can be found in many forms with different peptide and carbohydrate structures. Several commercial immunoassays are available to detect these hCG-related molecules. Quantitative results of different assays may vary remarkably depending on the antibodies used in the method.

In this work, two different commercial immunoassays were compared. Urine samples collected from male athletes and healthy male volunteers were analysed using both methods. In addition, urine samples from five male volunteers after intramuscular administration of hCG were also collected and measured.

### Experimental

#### Immunoassays

Two different commercial two-site immunochemiluminometric methods were used: Amerlite HCG-60 (Johnson&Johnson Clinical Diagnostics Ltd., USA) and ACS Total hCG (Chiron Diagnostics Corp., USA). The Amerlite HCG-60 assay uses a polyclonal capture antibody specific for  $\beta$ -subunit and a monoclonal labelled antibody specific for whole hCG. The measurement range of the Amerlite assay is 1 - 1000 IU/L and repeatability between series 3.4 - 9.0 CV%. The ACS Total hCG assay uses a monoclonal capture antibody and polyclonal labelled antibody specific for different epitopes of  $\beta$ -subunit. The measurement range of the ACS assay is 2 - 1000 IU/L and repeatability between series 5.0 - 8.1 CV%. All measurements were performed according to the manufactures' instructions. The assays were calibrated against the 1st IRP 75/537 standard of WHO.

#### Samples

*Urine samples from athletes:* Single untimed urine samples were collected for doping analysis from 148 male athletes. In addition, 528 urine samples were previously (1990 -1991) collected and analysed using the Amerlite assay.

*Urine samples from nonathletes:* Single untimed urine samples were collected from 45 healthy male volunteers (ages 15 - 49 years).

*Urine samples collected after intramuscular injection of hCG:* 5000 IU of hCG (Pregnyl, Organon) was administrated i.m. to five healthy male volunteers (ages 34 - 43 years). Spot urine samples were collected before administration and daily for 9 days after administration.

## Results and discussion

The hCG values of the 148 male athletes and the 45 healthy male volunteers were all below 1.0 IU/L as determined by the Amerlite assay (Table 1). The ACS assay gave higher results ranging from 1.4 to 6.6 IU/L (mean 2.8 IU/L, standard deviation 0.7 IU/L), possible due to interference of urinary matrix (Figure 1.). Most of the 528 samples, which have been analysed previously with the Amerlite assay, revealed hCG results below 1 IU/L. However, a few samples with high hCG values were found, possibly indicating abuse of hCG (Table 1). Measurement of the samples collected after hCG administration (5000 IU) showed that hCG excretion peaked one day after administration and remained elevated about 7 days. The urinary excretion of hCG was quite similar in all five subjects and the results obtained by both methods had a good correlation (Figures 2 and 3).

In conclusion, both immunoassays could be adopted as suitable screening procedures for the determination of abnormal urinary hCG concentrations in male athletes. When selecting an immunoassay for determination of hCG in urine as a part of doping control, each assay should be tested and validated carefully. Also decision limits for determining positive findings of hCG should be individually set for each assay.

## References

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TABLE 1. Distribution of hCG values in urine measured using the Amerlite assay.

hCG (IU/L)	≤ 1.0	1.1 - 2.0	2.1 - 3.0	3.1 - 4.0	4.1 - 5.0	Remarks
148 male athletes	100%	-	-	-	-	
45 male volunteers	100%	-	-	-	-	
528 male athletes (1990-1991)	92.6%	5.7%	0.4%	0.4%	0.2%	in addition, four remarkably high values (16.4, 26.6, 41.4 and 52.3 IU/l)

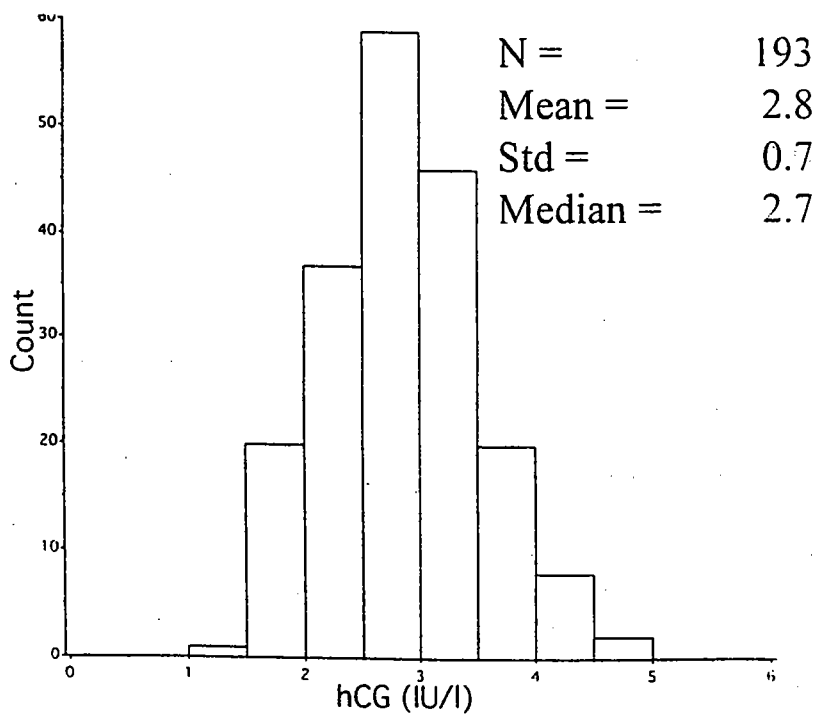


FIGURE 1. Distribution of hCG values in urine measured using the ACS assay. The data obtained from the 148 male athletes and the 45 male volunteers was combined.

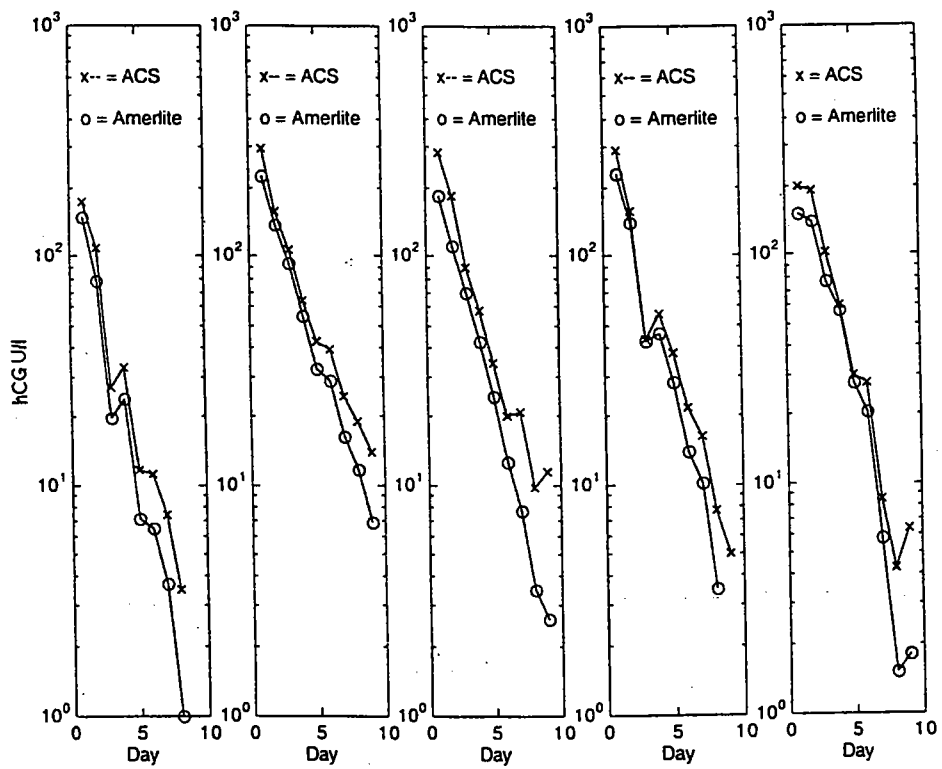


FIGURE 2. Urinary hCG values after administration of hCG. A single dose of hCG (5000 IU, i.m.) was injected to five male volunteers. Urines were collected daily for nine days.

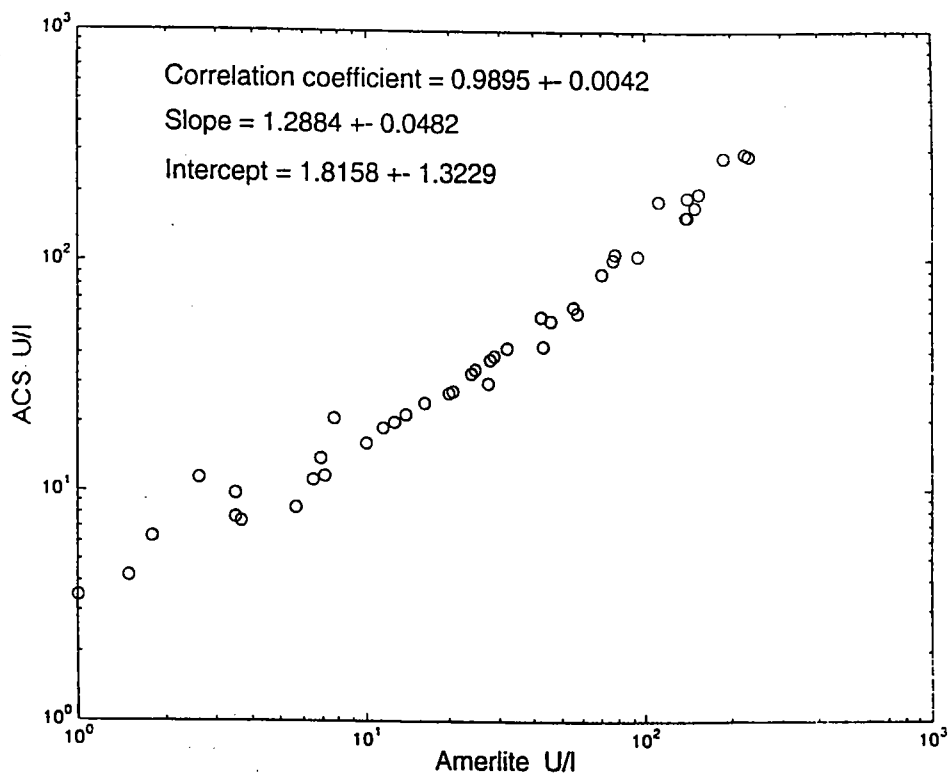


FIGURE 3. Comparison of the Amerlite assay and the ACS assay for hCG. The urines obtained from the excretion study were used as a testing material.