Comparison between GH assays. Serum GH cut-off levels by ECLIA performed in pharmacologhical stimulation tests in children with short stature

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INTRODUCTION:

- The diagnosis of growth hormone deficiency in children is based on clinical, auxological, imaging studies and biochemical criteria, which include maximum peak response to pharmacological stimulation tests (PhT).
- It is well known that GH concentrations vary remarkably depending on different factors, such as the assay method, matrix difference between standards and samples, the IRP IS used in calibration, specificity of antibodies, and interference with endogenous GH binding proteins (GHBPs).
- In our population, the proposed cut-off value of serum GH PhT is 4.70 ng/mL measured by ICMA using the IRP 98/574; however, no cut-off values have been obtained using ECLIA with the same IRP.

AIMS

To define GH cut-off level by ECLIA in our population and to compare the concentrations measured by ECLIA and ICMA.

SUBJECTS AND METHODS

- 192 children with short-stature were analyzed
  F: 58 M: 134 Chronological age: 0.8 to 16 years
- 245 PhT were performed Arginine n: 175 Clonidine n: 70

Statistical analysis:
Linear regression analysis and Phi coefficient of association.

- 1120 GH samples were measured by ICMA (Immulite 2000) and ECLIA (Cobas e 601) using the IRP 98/574.

Table 1: Analytical characteristics of ECLIA and ICMA methods.

Method	Functional Sensitivity	Analytical Sensitivity	VC%(5ng/ml)	Reference Preparation
ECLIA	0.18	0.035	5.67	IRP 98/574
ICMA	0.11	0,055	6.84	IRP 98/574

RESULTS

❖ No statistically significant differences were observed between GH values by sex and PhT.

Distribution of GH values: ECLIA / ICMA ratio vs ICMA

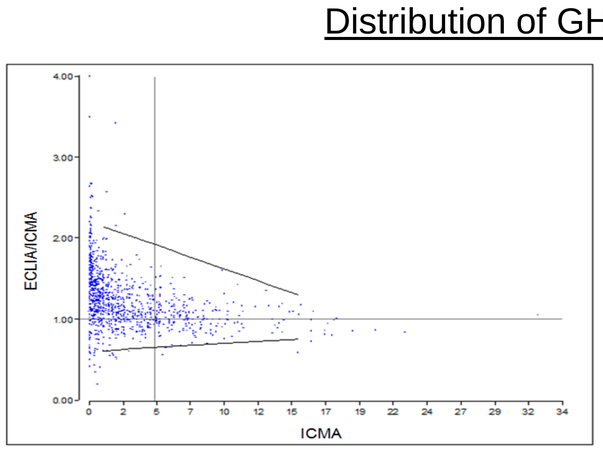

Fig 1: Slope for ratio = 1 and 95% confidence interval (quantile regression).

❖ At low GH concentrations, values measured by ECLIA are higher than those measured by ICMA; the bias between methods is reduced with increasing GH levels, and the ratio approaches slope1.

Table 2: Means and standard deviations for the ECLIA-ICMA difference according to increasing ranges of ICMA concentrations.

ICMA ng/mL	Δ Mean (ECLIA-ICMA)	SD
(0.05 – 2]	0.0186	0.348
(2 – 4]	0.422	0.745
(4 – 6]	0.415	1.035
(6 – 8]	0.277	1.097
(8 – 40]	-0.091	2.020

❖ The difference decreases with increasing ICMA concentration.

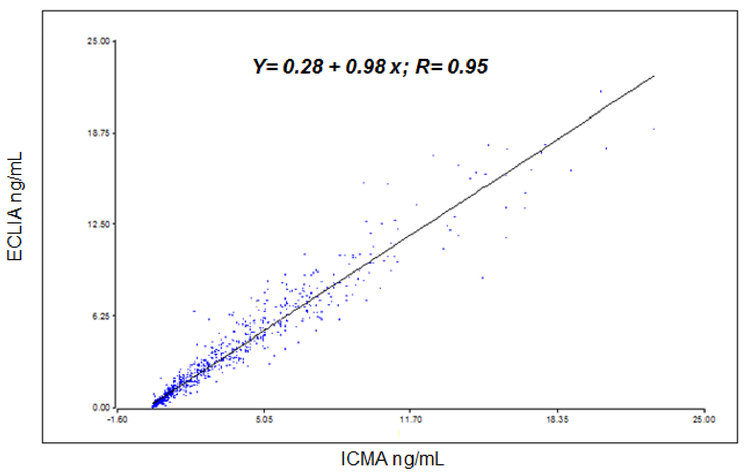

Fig 2: Linear regression analysis of ICMA vs ECLIA showing highly significant linearity

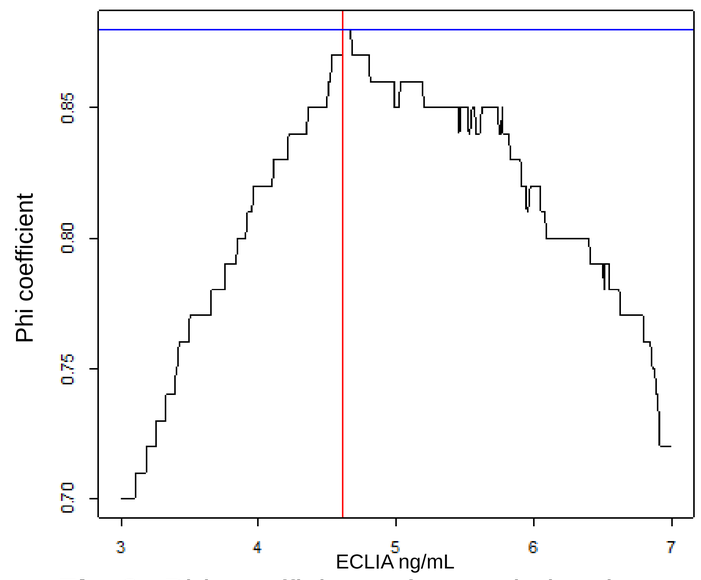

Fig 3: Phi coefficient of association between ICMA and ECLIA

❖ The cut-off value for ECLIA was established calculating, for several cut-offs values between 3 and 7 ng/mL (in steps 0.01 ng/ml), the phi coefficient of association between the classifications obtained by ICMA and ECLIA. The maximum phi (0.88) was attained at 4.65 ng/mL.

Table 3: Maximum response to PhT. Discordant values between the diagnoses by ECLIA and ICMA.

ICMA ng/mL	ECLIA ng/mL
3.80	5.72
4.40	4.71
4.40	4.78
3.70	5.12
3.00	5.02
4.40	5.46
4.20	5.00
5.00	4.18
6.00	4.03
5.30	4.02

No response by ICMA

No response by ECLIA

❖ We found 96% agreement in the interpretation of PhT using the cut-off for each method. Of the 245 tests performed, 100 did not show any response by either method.

❖ 4 % disagreement corresponds to 10 PhT.

DISCUSSION

- GH measurements by ICMA and ECLIA using the IRP 98/574 were highly correlated. The adoption of a single standard has improved the comparability of the methods to determine GH.
- The establishment of cut-off level by ECLIA in our population help to a consistent interpretation of the results and would contribute to the improvement of the diagnostic complexity of GH deficiency in children.

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