GH harmonisatie

Eef Lentjes
namens de SKML-sectie endocrinologie

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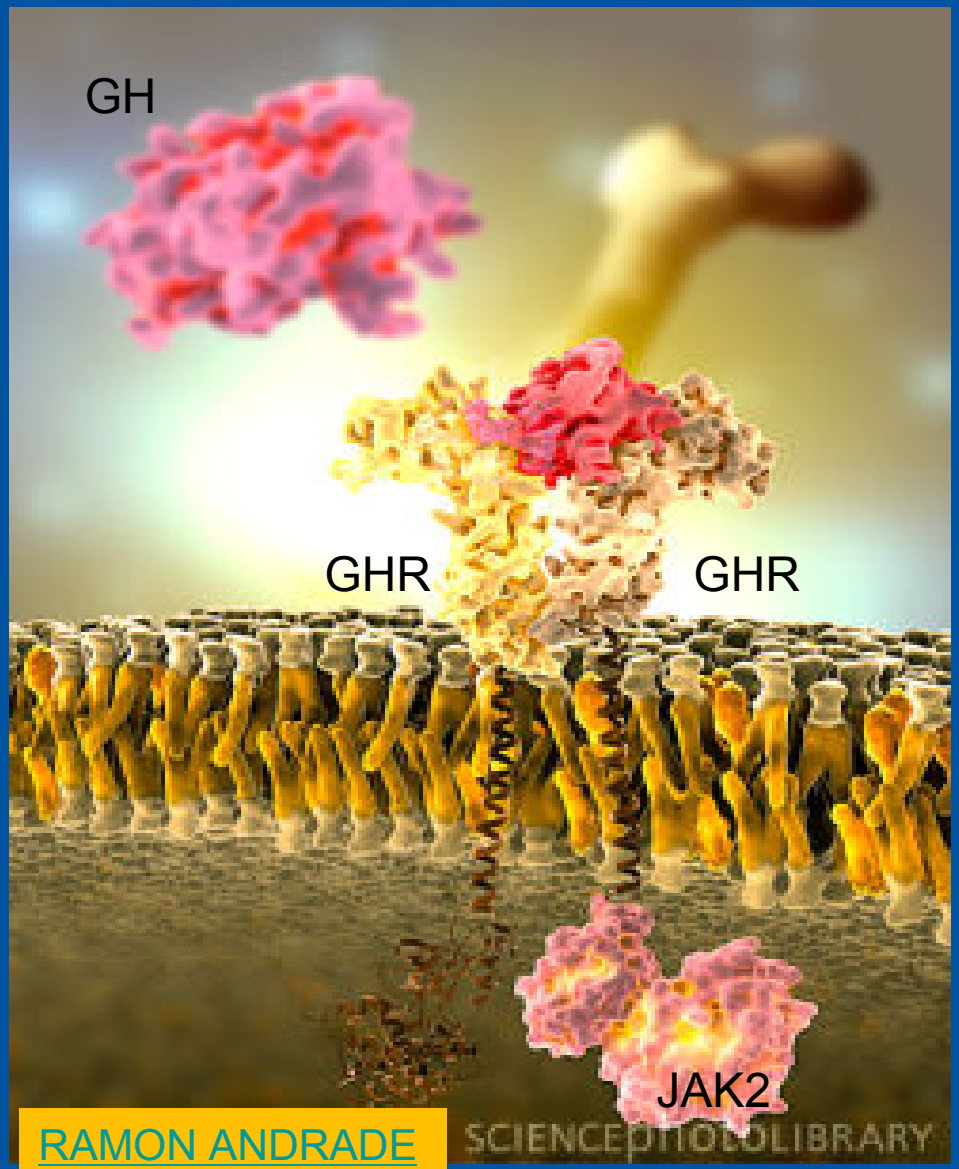

Gebaseerd op presentatie van Alec Ross

Growth hormone harmonisation in the Netherlands: reduction of between-lab variation by introduction of a single reference sample

(SKML 2006)

Primaire structuur groeihormoon & varianten

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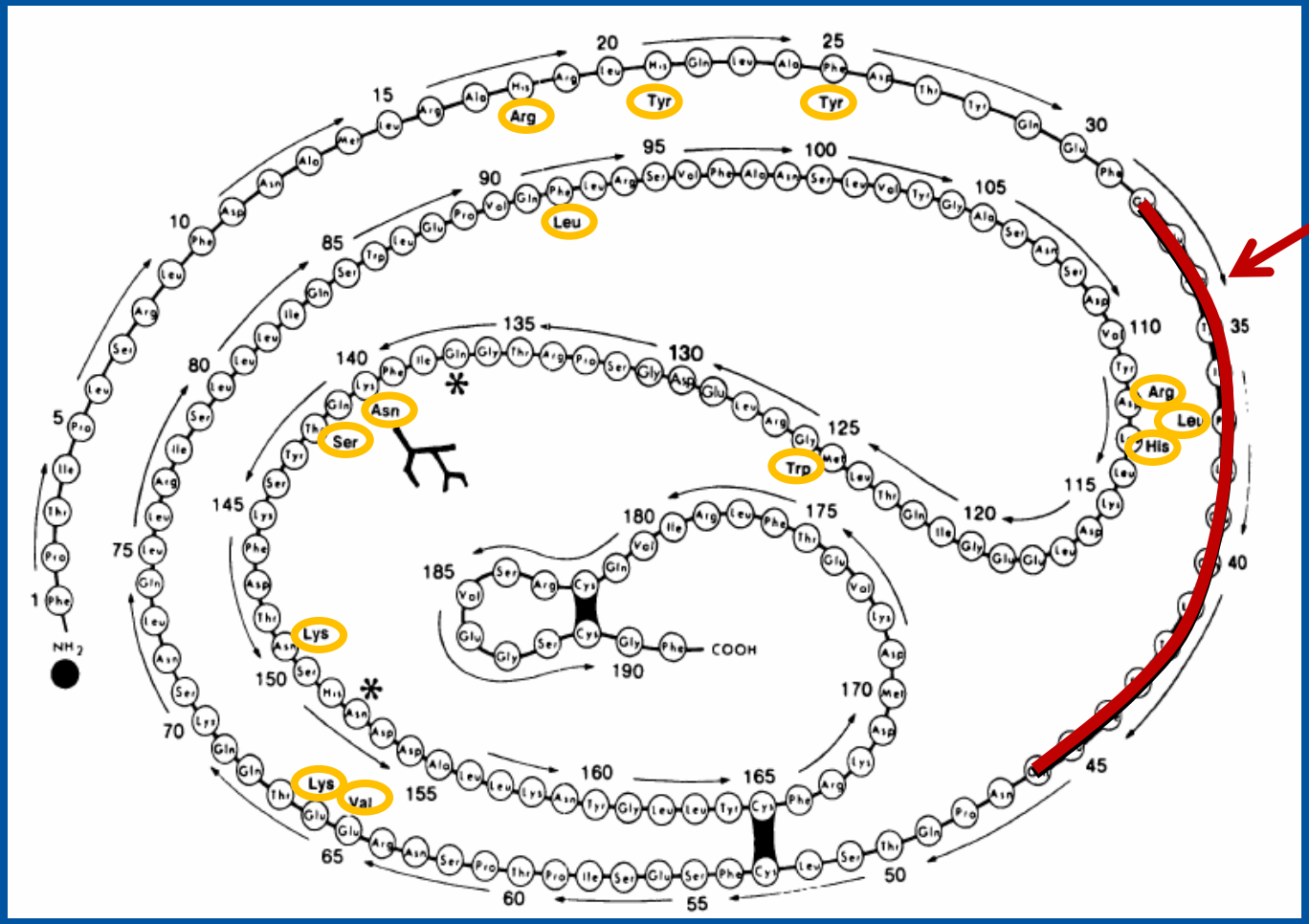

22 KD: 191 az
20 KD: ex 32-46

O: placentair GH
: glycosylering (placent. GH)

★: deamidering
●: az1-acylering

Baumann 1991; Endocr Rev

Major constituents of serum hGH

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Monomers:			
22K			
free		21%	
bound		22%	
total 22K			43%
20K			
free		5.5%	
bound		2.5%	
total 20K			8%
Desamido- forms			5%
Oligomers:			
22K-dimers	total		20%
20K-dimers	total		7%
higher			15%
Fragments:			0-2%

Baumann 1991; Endocr Rev

Oorzaak van variatie in GH resultaten

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- GH vormen in circulatie
  - 22 en 20 KDa, fragmenten GH(1-43), GH (44-191)
  - Monomeren, dimeren en oligomeren, desamido-GH, N-acetyl-GH
- GH-binding protein: 50% GH gebonden
  - Invloed van GHBP o.a. door Mab's en korte incubatietijden op analysers (Negatieve bias door afscherming van antibody bindingsplaats of epitopen)
  - Variatie in concentratie door voeding en metabolisme
- Calibratoren
  - hypofyse extracten: IS 66/217 (1969) en IS 80/505 (1982)
  - Recombinant 22 KDa: IS 88/624 en IS 98/574 (>96% 22 KDa)
  - Matrix van calibrator
- Methoden
  - Poly-monoclonaal, competitief – sandwich
  - Gebruik van diverse omrekeningsfactoren van µg/L → mU/L

Interferentie

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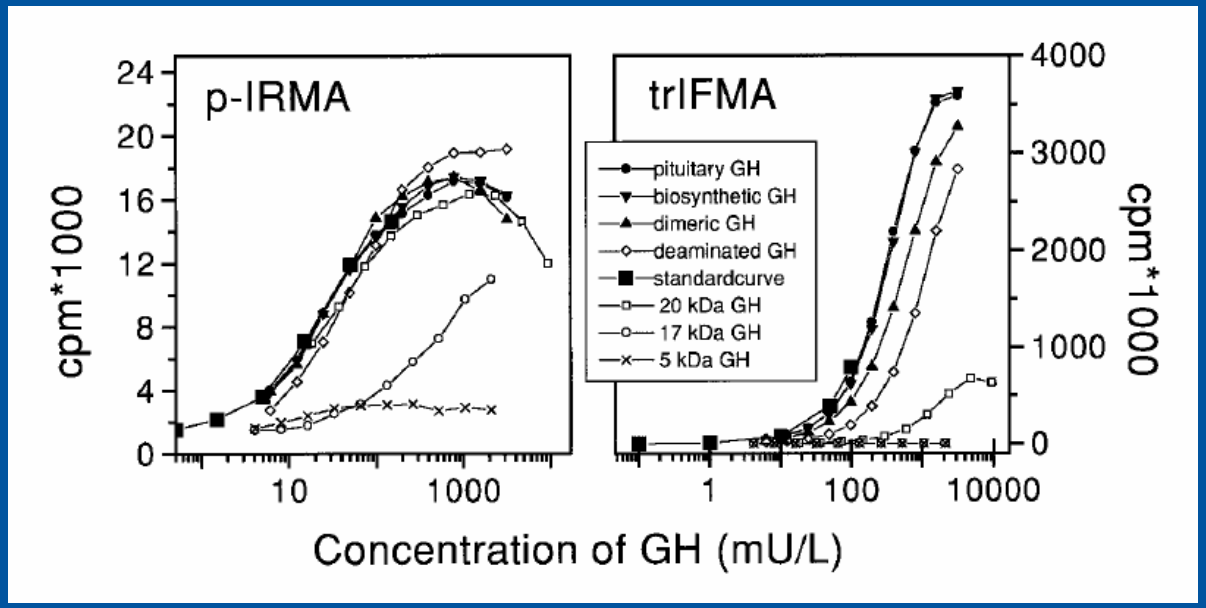
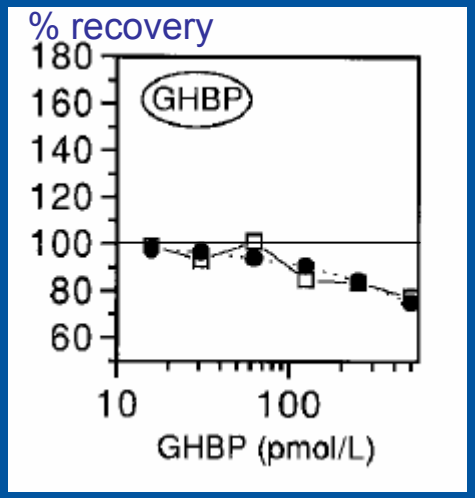
Kruisreacties isovormen GH
Pharmacia polyclon/ Delfia monocl



Polyclonaal Ab (●)
Monoclonaal Ab (□)



Jansson Clin Chem 1997

Interferentie door Pegvisiomant

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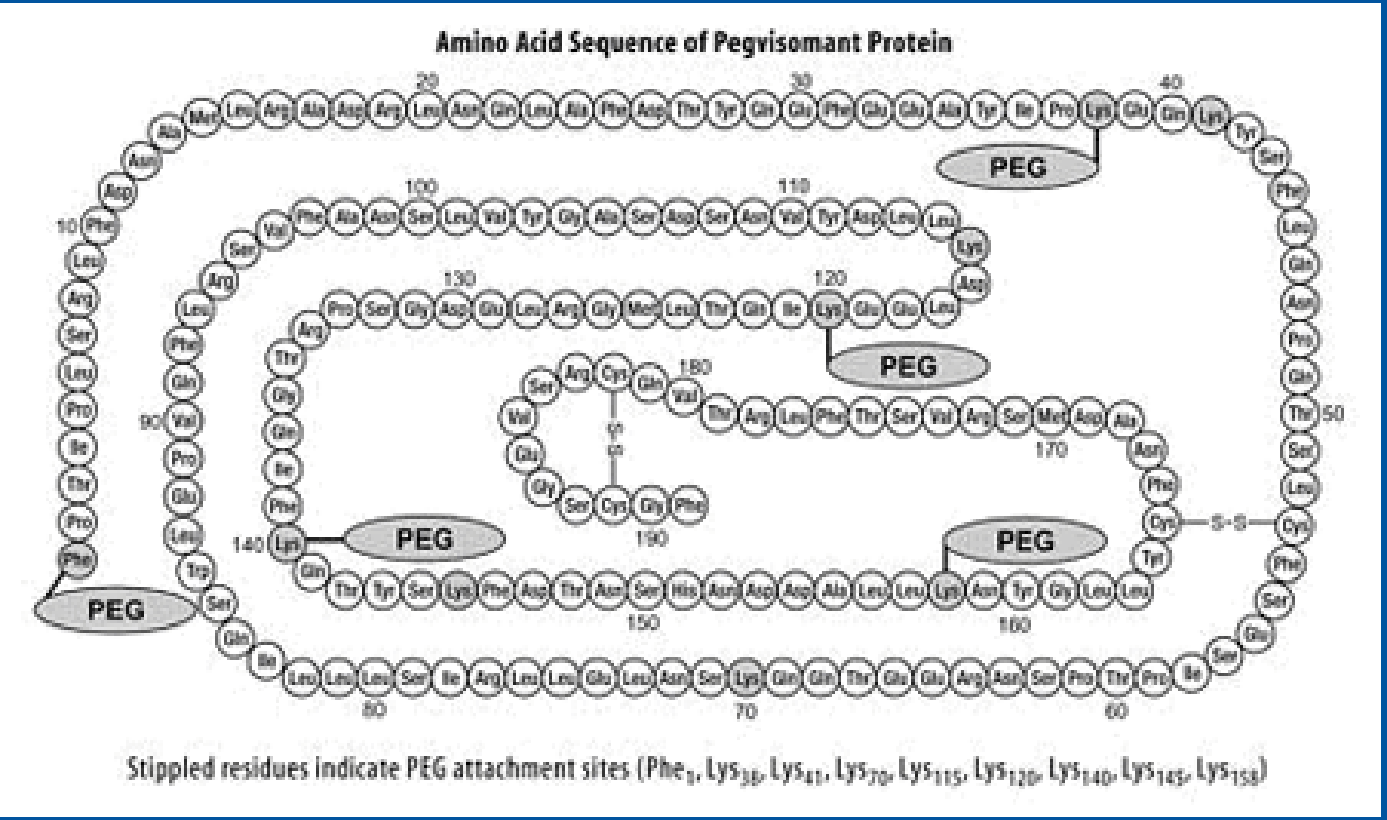

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4. UNITAGE

1.95 mg per ampoule
(somatropin + somatropin-related impurities)

3.0 International units per mg Somatropin

Uncertainty: The International Unit of 98/574 is assigned without uncertainty. Where required, the uncertainty of the ampoule content of 98/574 may be considered to be the coefficient of variation of the fill volume, which was determined to be 0.07%.

A World Health Organization International Laboratory For Biological Standards

National Institute for Biological Standards and Control

1470

The Institute's UKAS accreditation relates only to our programme of testing for biological medicines.
Full details can be found on our Schedule of Accreditation, which is available on request.

STANDARDIZATION

The assay is calibrated to the NIBSC 2nd IS 98/574 for Somatropin (22kD recombinant DNA derived materials). The previous calibration (Catalog No. 62-7006) was based upon the older WHO 1st IS 80/505 reference preparation (pituitary derived hGH). To convert 2nd IS 98/574 results to the older 1st IS 80/505 (Catalog No. 62-7006) divide the results in ng/mL by the factor 0.56.

To convert result in ng/mL to µIU/mL, multiply the result by the factor 3.0 µIU/ng, per WHO documentation.

Conversion Factor:

ng/mL × 2.4 → mIU/L
WHO NIBSC 2nd IS 98/574
(For kit lots 206 and up)

ng/mL × 2.6 → mIU/L
WHO NIBSC 1st IS 80/505
(For kit lots 205 and below)

Vanaf lotnr 109 (gebruik MoAb Imm 1000) ng/ml x 3.0 → mU/L WHO 98/574

EQAS: Ringversuch 2006 (RfB)

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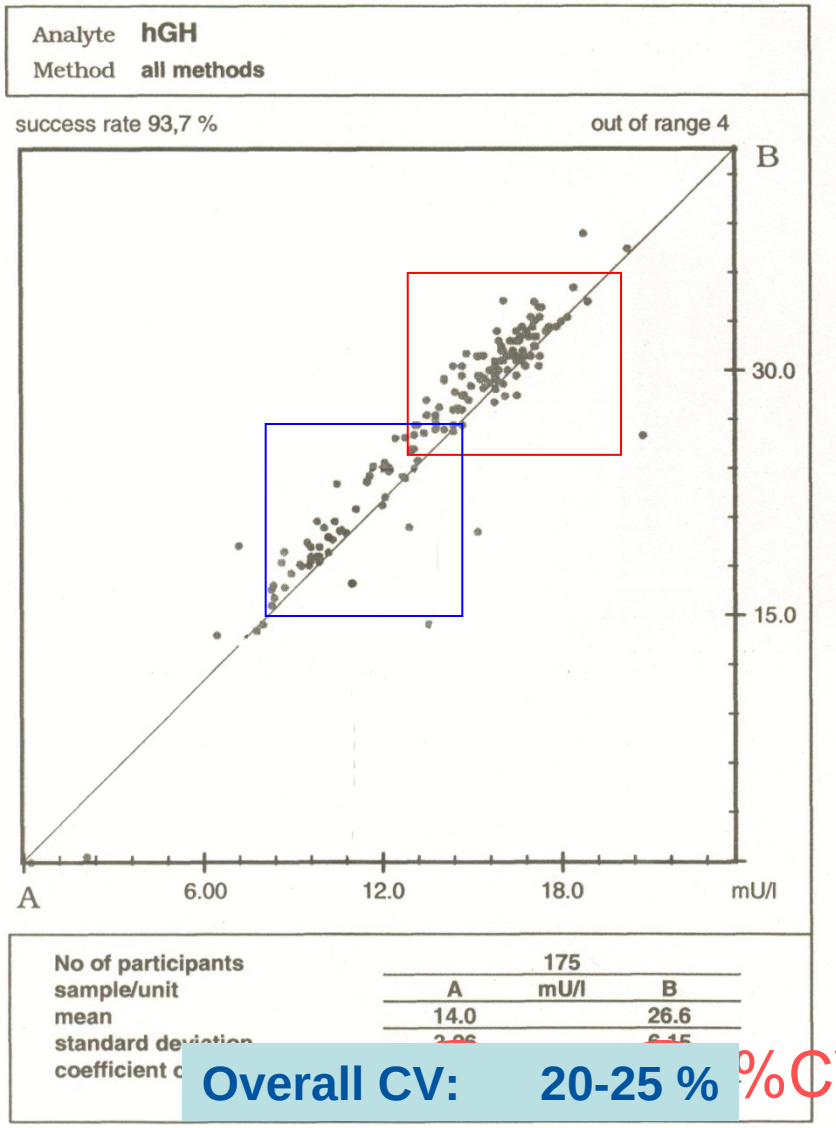
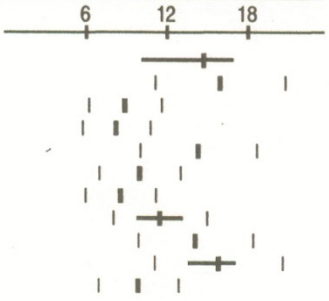


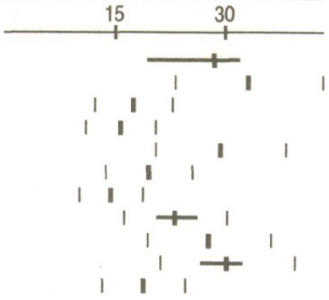
Sample A mU/l

M	Kit	N	Min	16.P	50.P	84.P	Max
Alle		175	0.250	10.2	14.9	17.0	49.9
1	36	3	14.9		16.1		17.3
Scher2		3	8.74		8.96		24.1
1	53	4	7.80		8.31		8.39
1	76	7	12.1		14.4		49.9
1	111	6	7.20		10.1		15.2
2	66	3	6.45		8.62		13.6
3	91	11	9.50	9.78	11.5	13.2	13.9
4	13	4	13.8		14.1		15.6
4	44	107	9.90	13.6	15.9	17.1	35.1
4	77	5	9.60		9.90		10.4



Sample B mU/l

M	Kit	N	Min	16.P	50.P	84.P	Max
Alle		175	0	19.5	28.7	32.1	90.0
1	36	3	31.1		33.3		34.3
Scher2		3	16.7		17.6		46.4
1	53	4	14.1		15.9		16.6
1	76	7	24.4		29.5		90.0
1	111	6	18.9		19.7		23.1
2	66	3	13.8		14.5		18.2
3	91	11	19.5	20.7	23.3	26.2	27.8
4	13	4	26.7		27.8		30.0
4	44	107	18.6	26.7	30.3	32.4	70.5
4	77	5	18.3		18.9		19.7



Other kits (number):
1 23(2), 1 43(2), 1 77(2), 1 79(1), 1 99(2), 2 35(1), 2 54(1), 2 111(2), 3 04(1), 3 54(2), 4 62(2), 4 262(2), 4 99(1), 4 111(1),

Median method 91:
A 11.5, B 23.3
Median method 44:
A 15.9, B 30.3
Systematic difference: 27%!

Overall CV: 20-25 % %CV

CBO consensus “Diagnostiek kleine lichaamslengte bij kinderen” 1996

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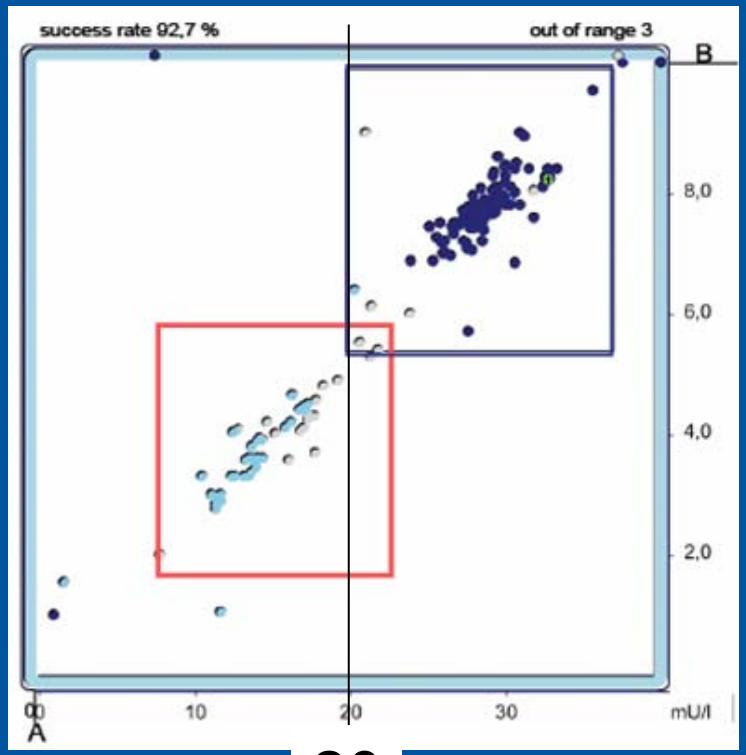
…..een maximale groeihormoon-serumconcentratie < 20 mE/l na uitvoeren van 2 verschillende provocatietests, waarbij voorbehandeling met geslachtshormonen (`priming') bij prepuberale kinderen in de puberale leeftijd dient te worden toegepast;



Toekenning van GH wordt Bepaald door gebruikte methode
→Verzoek van Ned Groeistichting om verschillen te verkleinen

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Ook internationale richtlijnen gebruiken cutoff waarden

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Utrecht

European Journal of Endocrinology (2007) **157** 695–700 ISSN 0804-4643

2007

CONSENSUS STATEMENT

Consensus guidelines for the diagnosis and treatment of adults with GH deficiency II: a statement of the GH Research Society in association with the European Society for Pediatric Endocrinology, Lawson Wilkins Society, European Society of Endocrinology, Japan Endocrine Society, and Endocrine Society of Australia

Ken K Y Ho on behalf of the 2007 GH Deficiency Consensus Workshop Participants

ITT and glucagons test: a peak GH< 3 µg/l (=9 mU/L)

GHRH/arginine:

BMI <25 kg/m2:	a peak GH<11 µg/l (=33 mU/L)
BMI 25–30 kg/m2:	a peak GH<8 µg/l
BMI >30 kg/m2:	a peak GH<4 µg/l

The GH Res Soc advocates the use of recombinant 22 kDa GH calibrator (International Reference Preparation (IRP) 98/574) in all GH assays.

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JCEM 2011 Endocrine Society (2006 update)

SPECIAL FEATURE

Clinical Practice Guideline

Evaluation and Treatment of Adult Growth Hormone Deficiency: An Endocrine Society Clinical Practice Guideline

Mark E. Molitch, David R. Clemmons, Saul Malozowski, George R. Merriam, and Mary Lee Vance

Cutoff points for ITT: 5.1 µg/L and for GHRH/arg: 4.1 µg/L (Biller JCEM 2002)
Use different GHRH/arg cutoff points according to BMI.

JCEM 2011 Endocrine Society

CONSENSUS STATEMENT

Biochemical Assessment and Long-Term Monitoring in Patients with Acromegaly: Statement from a Joint Consensus Conference of The Growth Hormone Research Society and The Pituitary Society

"For each GH assay, normative data for glucose-suppressed GH concentrations are necessary for conclusions about adequate control in individual patients. To define restoration of normal neuroregulation of GH secretion, glucose suppression of GH should be measured. This may correspond to levels as low as 0.3 µg/liter in a two-site assay with monoclonal antibodies."

Situatie nu ?
Ringversuch 2011 (RfB)

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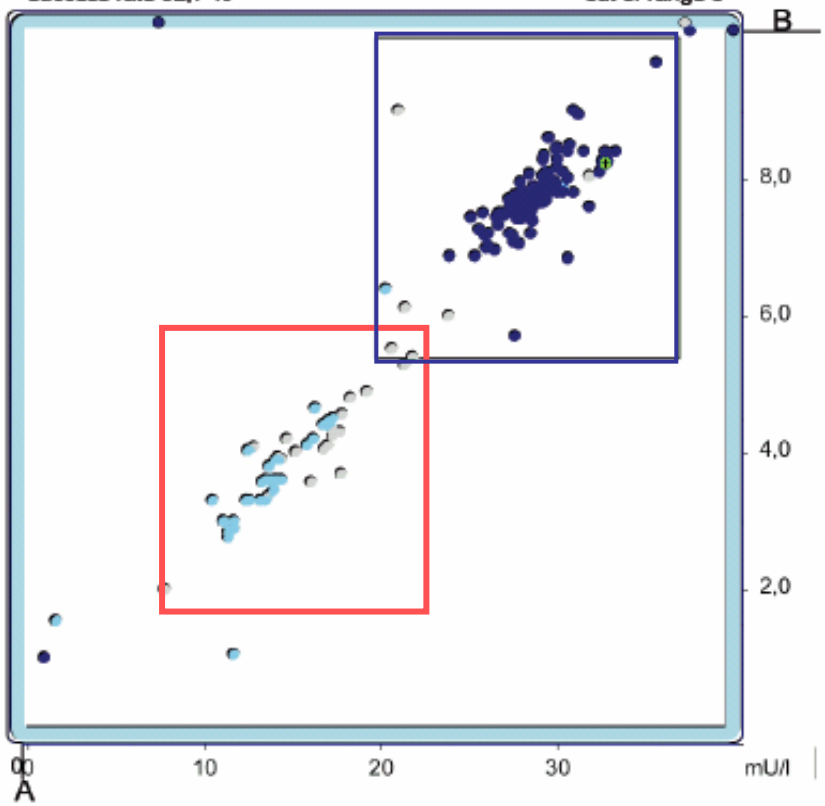
Analyte hGH
Method all methods

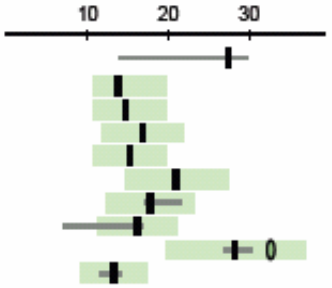


No of participants		179	
sample/unit	A	mU/l	B
mean	23.6		6.34
standard			
coefficien			

2010-2011: 13-36 %CV

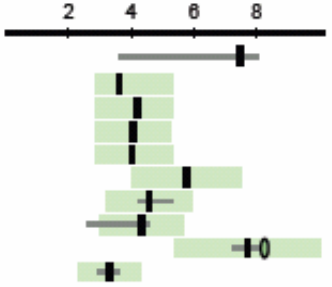
Sample A [mU/l]

M	Kit	N	Min	16.P	50.P	84.P	Max
Alle		179	1.00	13.9	27.4	29.9	40.2
1	77	3	13.7		13.8		14.2
1	99	3	14.7		14.7		21.0
1	111	5	14.4		16.8		18.3
1	143	3	7.80		15.2		17.1
2	66	4	17.8		21.0		23.9
3	91	9	17.0	17.1	17.8	21.5	21.8
4	44	111	1.00	27.0	28.3	30.3	40.2



Sample B [mU/l]

M	Kit	N	Min	16.P	50.P	84.P	Max
Alle		179	1.00	3.60	7.47	8.04	27.9
1	77	3	3.36		3.60		3.93
1	99	3	4.20		4.20		9.00
1	111	5	3.57		4.05		4.80
1	143	3	2.00		4.01		4.47
2	66	4	3.69		5.76		6.12
3	91	9	4.10	4.19	4.56	5.33	5.40
4	44	111	1.00	7.26	7.70	8.25	27.9



The deviation of your results from the median of the corresponding sub-collective (kit) is: A 15 %, B 7.14 %

Andere Kits (Anzahl):
1-41(1), 1-76(1), 1-92(1), 4-23(1), 4-30(2), 4-40(1),

Median method 91:
A 17.8, B 4.56
Median method 44:
A 28.3, B 7.70
Systematic difference: 40%!

Kunnen de verschillen kleiner?

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- Standaardiseren is alleen mogelijk voor enkelvoudige en eenduidige componenten als T4 en cortisol
  - Standaarden met true values
  - elimineren van interferentie wordt dan de uitdaging
    - LCMS-MS
    - extractie-immunoassay; chromatografie-immunoassay
- Voor hormonen die in meerdere vormen circuleren en ook biologische aktief zijn is standaardiseren niet mogelijk. Bv GH, LH, TSH, prolactine
- Harmonisatie is mogelijk als methoden goed correleren.
  - Kan afhankelijk zijn van ziektebeeld: verhouding isovormen
  - Nier-, leverfunctiestoornissen: klaringsverschillen van isovormen

Eerste pilot (1994) 9 deelnemers, 14 monsters

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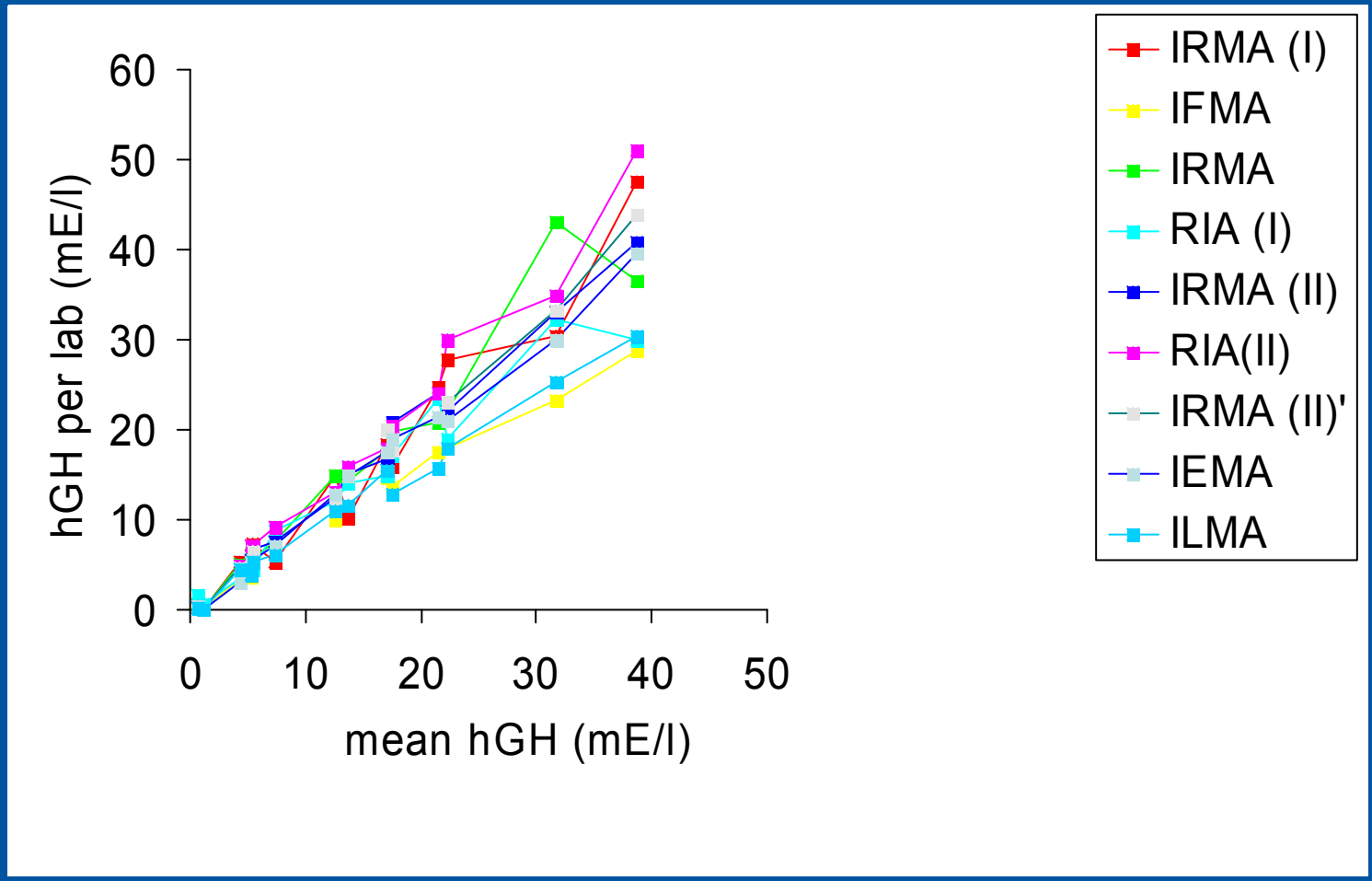
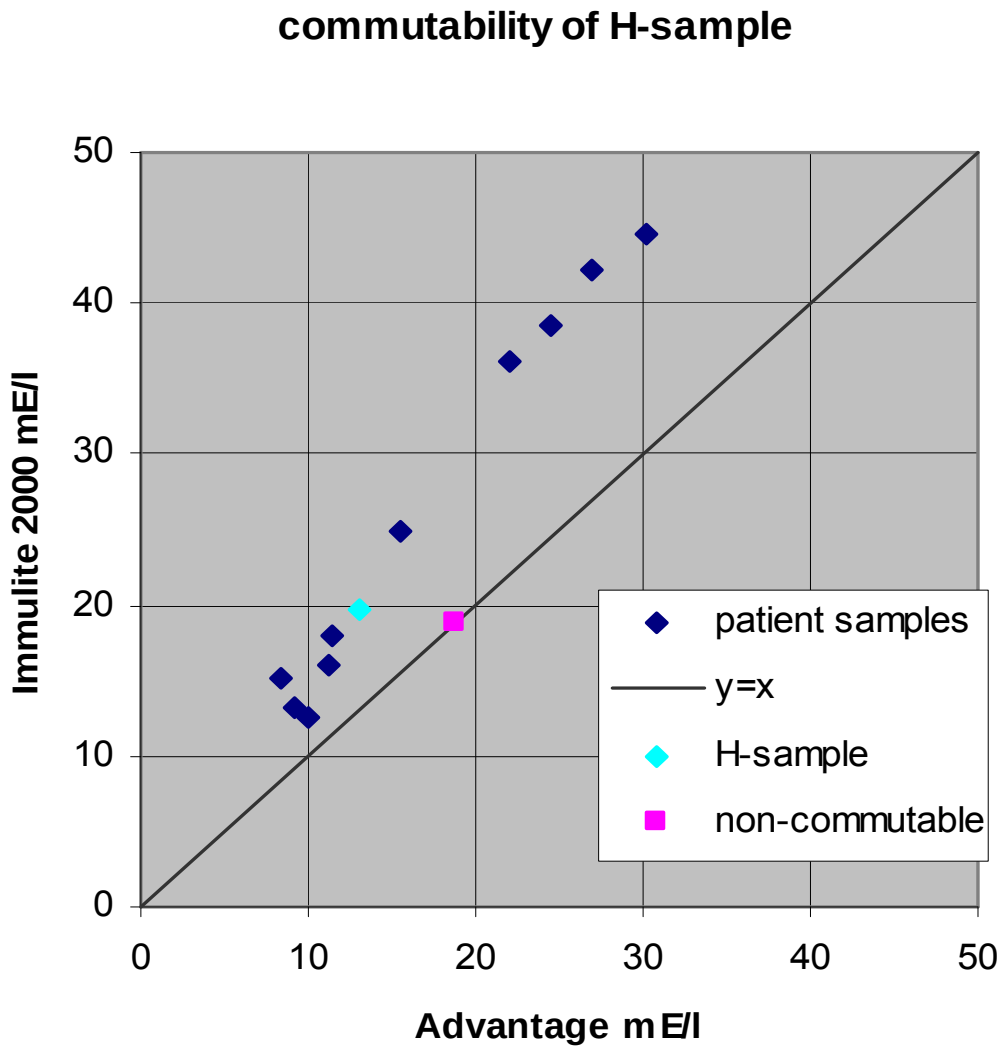


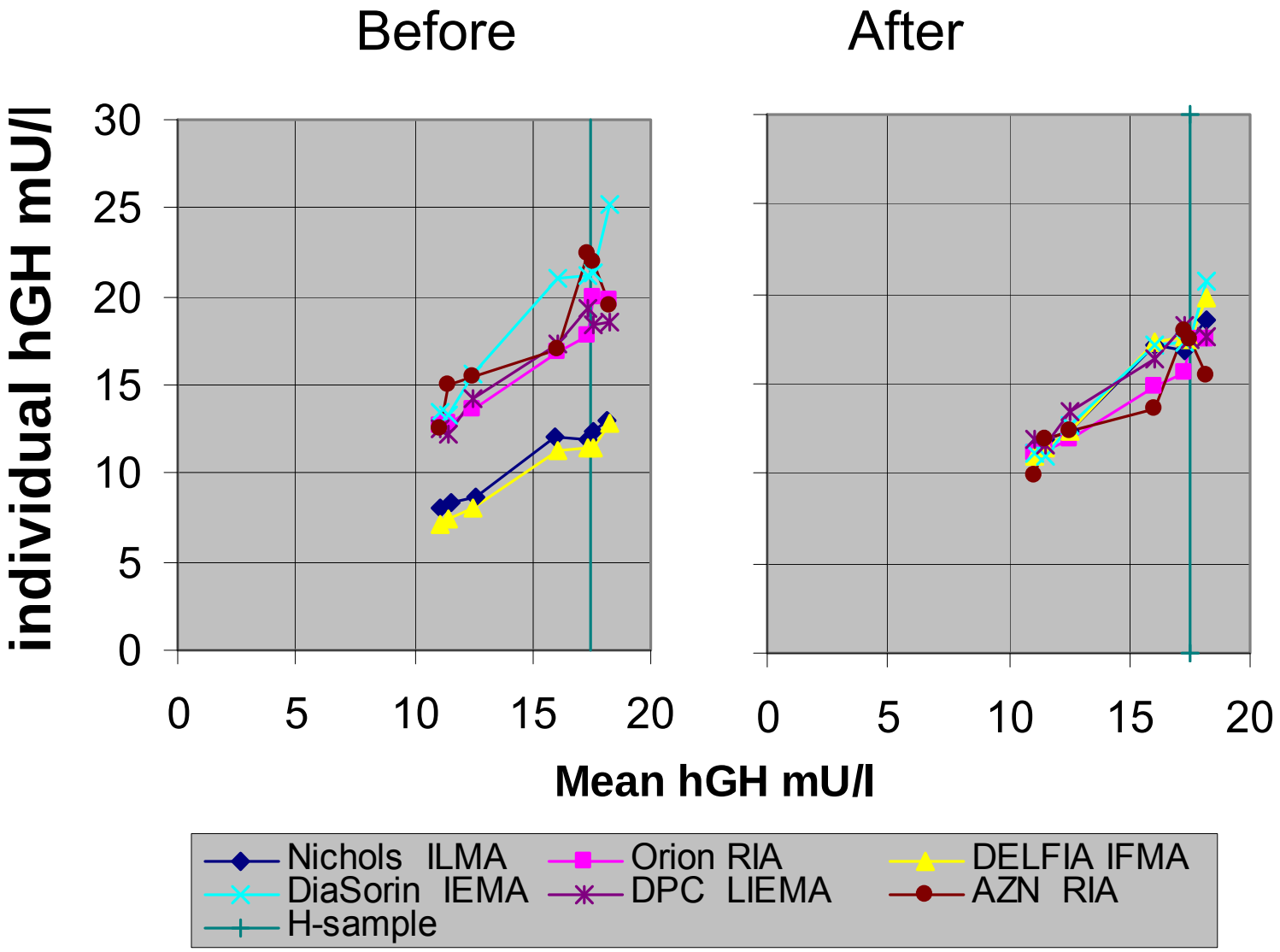


Commuteerbaar H monster (1999)

- Serum van gezonde donoren, na inspanning

- Consensuswaarde = gemiddelde van alle methoden (17.5 mE/L)

Pilot:effect of harmonization



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‘Twin’ study for evaluation of GH harmonization:

- Pairs of labs that use different GH assays are formed
- These pairs exchange patient samples with GH levels between 10 and 30 mU/l
- Both labs measure GH in these samples and the H-sample
- The harmonization correction is performed.

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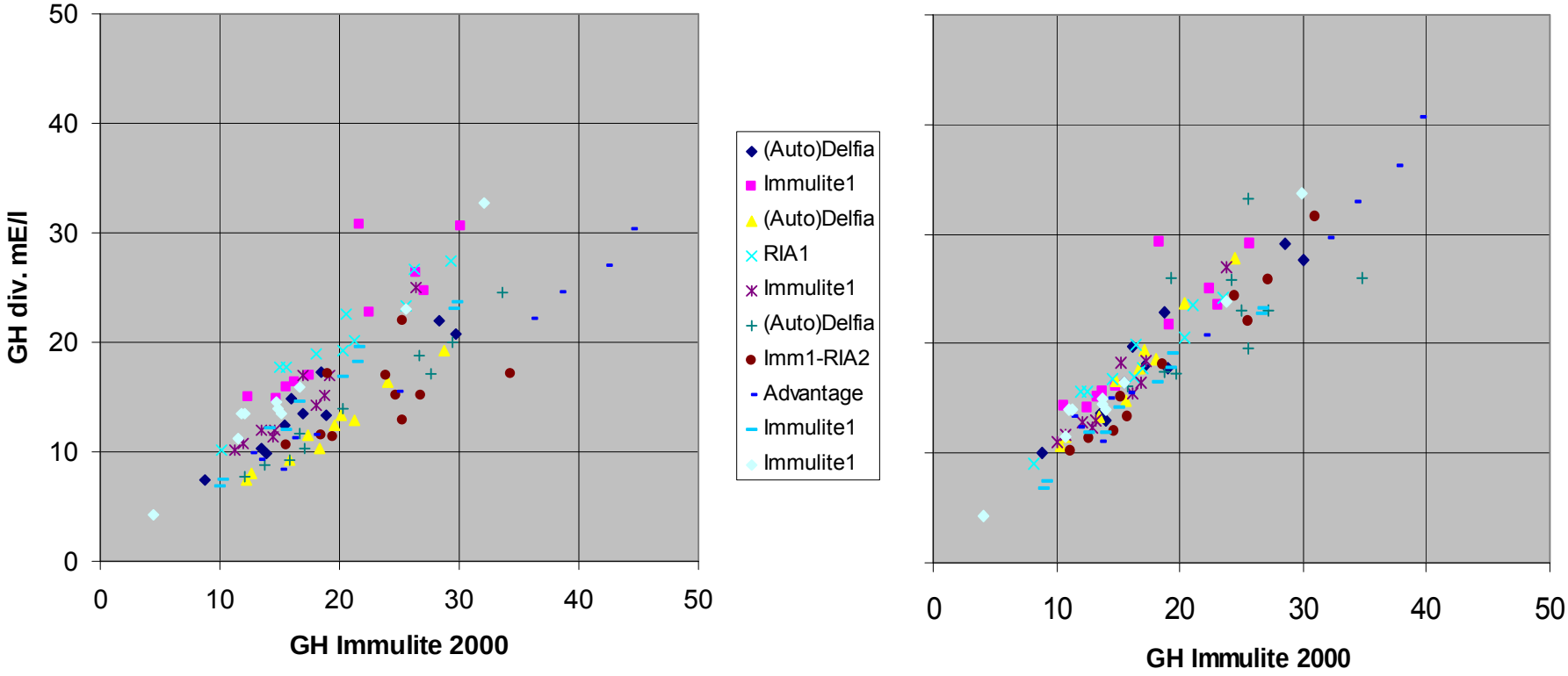
GH twin study 2004



Avg. corrected between-lab CV,(including previous twin study)

Direct: **21%** (22%)

Harmonized: **6**% (8%)

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SKML LWBA ronde 2004/4

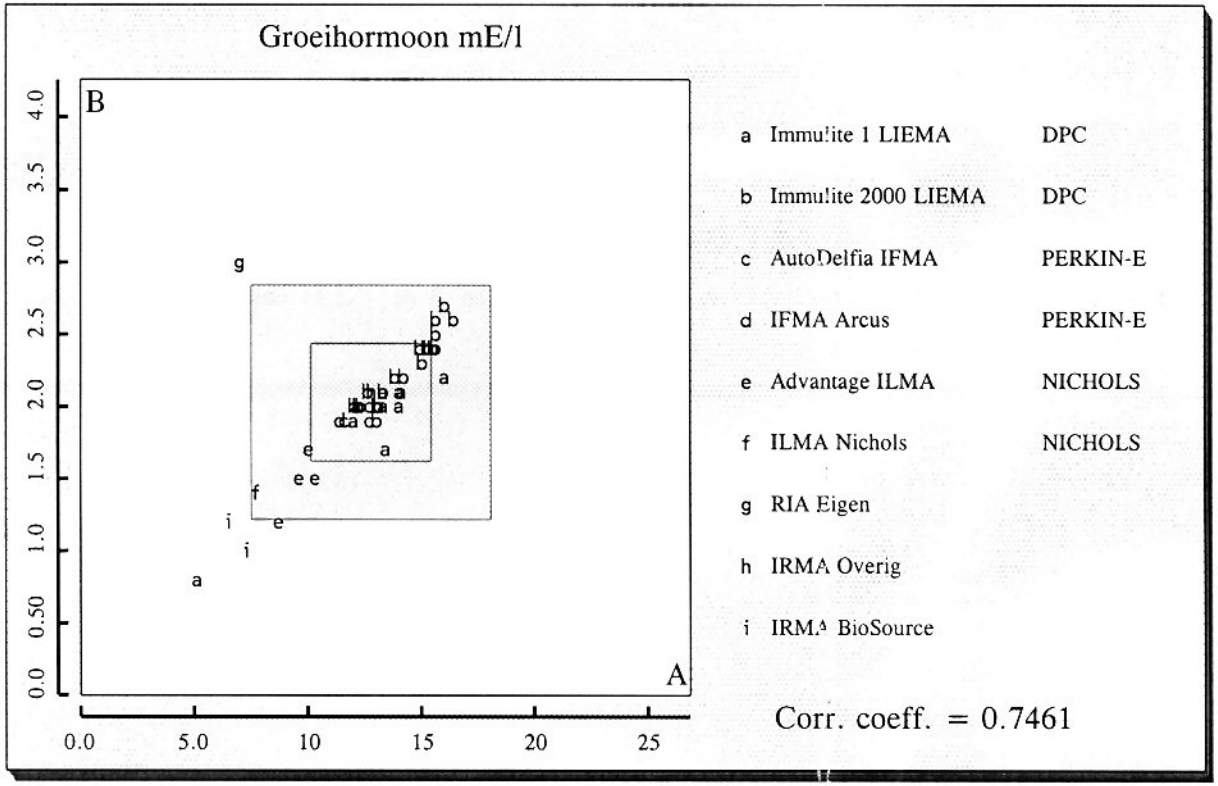
Groeihormoon mE/l

Toevoeging: 11.6 mE/l
Aantal Kits/Methoden: 12

20.7%

20.0%

Methode	Groep	N	A Gem.	SD	CV	N	B Gem.	SD	CV %	Rec %
TOTAAL		46	12.8	2.64	20.7	44	2.03	0.405	20.0	91.4



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SKML LWBA ronde 2005/3

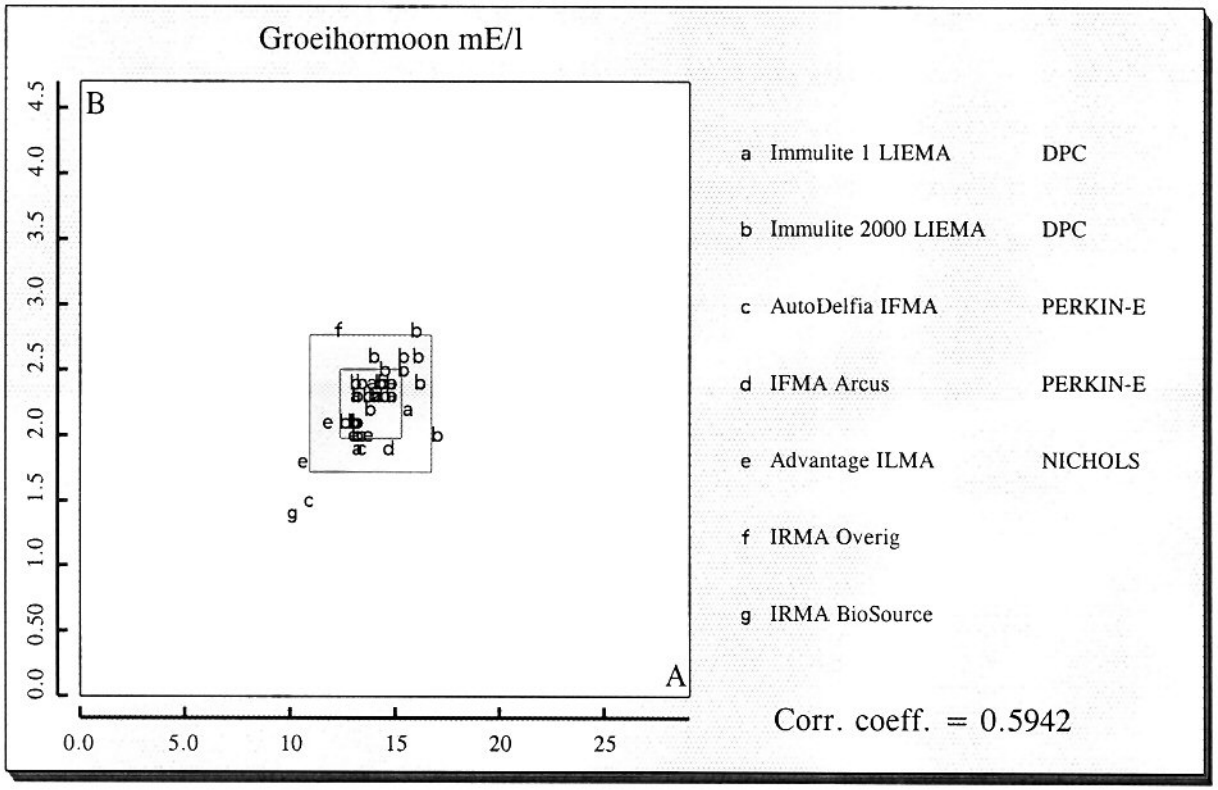
Groeihormoon mE/l

Toevoeging: 12.4 mE/l
Aantal Kits/Methoden: 9

10.5%

11.7%

Methode	Groep	N	A Gem.	SD	CV %	N	B Gem.	SD	CV %	Rec %
TOTAAL		45	13.9	1.46	10.5	43	2.24	0.262	11.7	93.7



Resultaten sinds start harmonisatie

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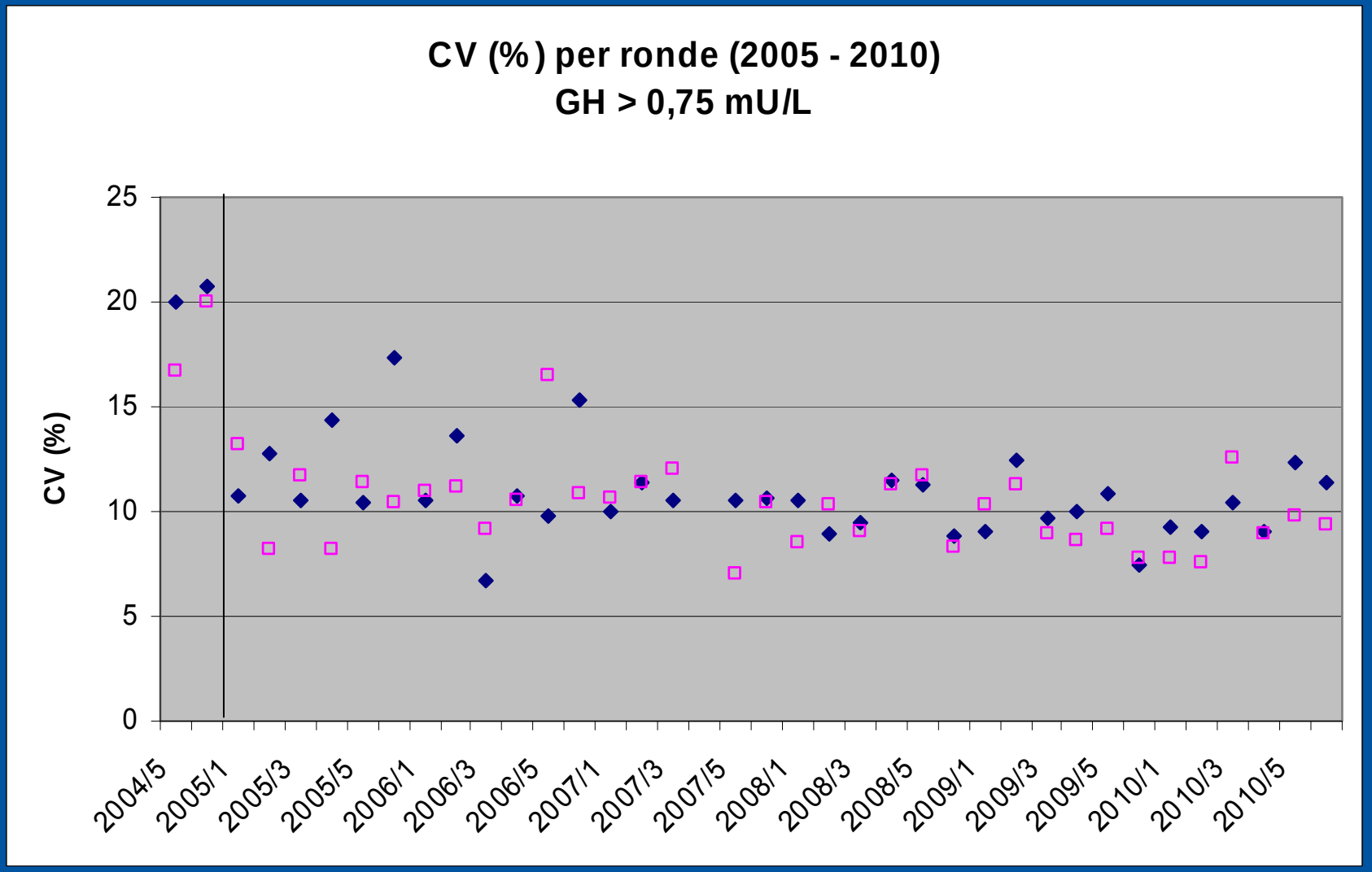

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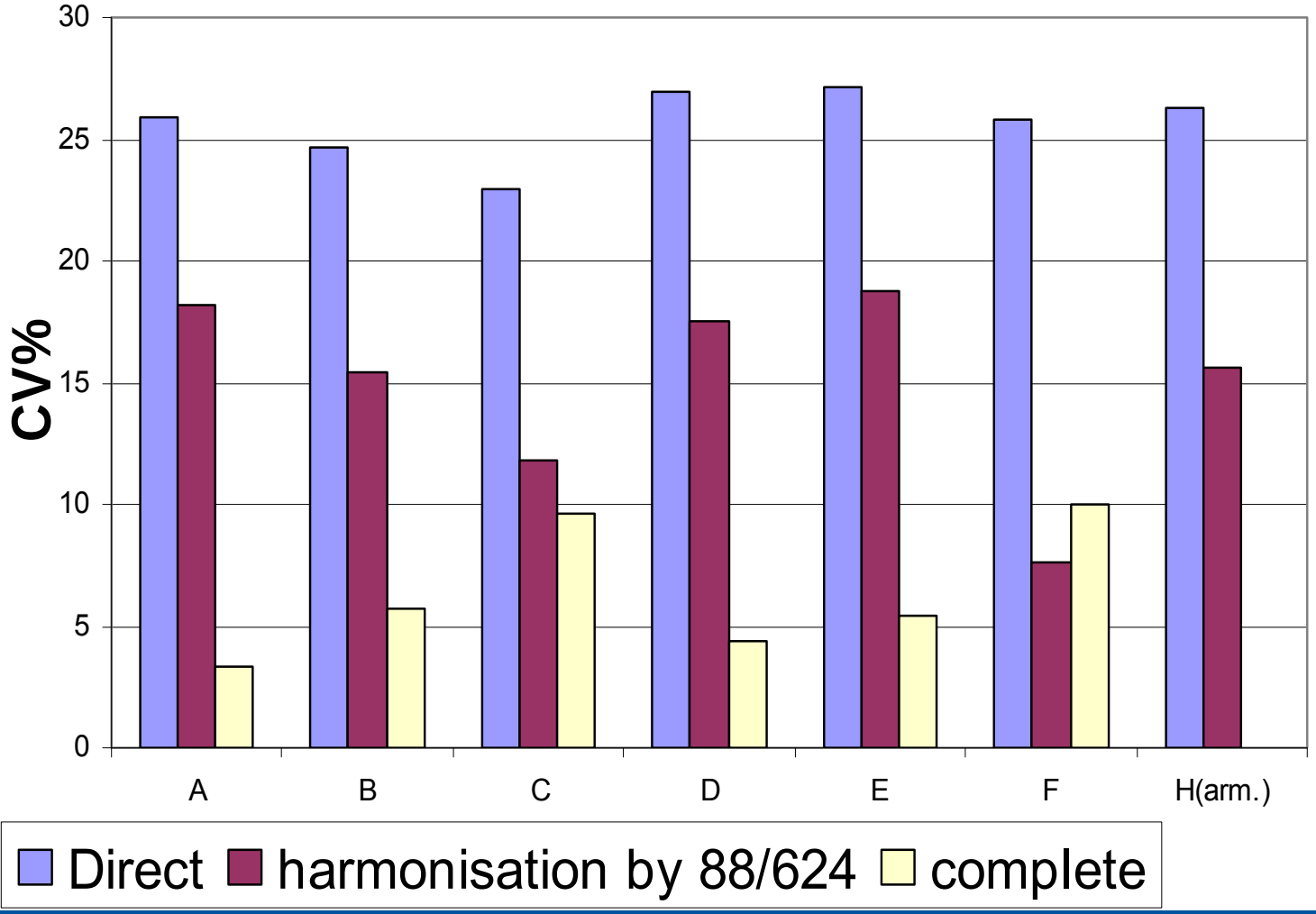
EQA results do not realistically represent the effect of harmonisation, as QC samples are spiked with recombinant GH

Conversely, attempts to harmonize just by employing the same recombinant standard, as recommended by the British Association for Clinical Biochemists (Eur. J. of Endocrinology 2006;155:1-2) are bound to give sub-optimal results.

Effect of partial and complete harmonisation on between-lab cv



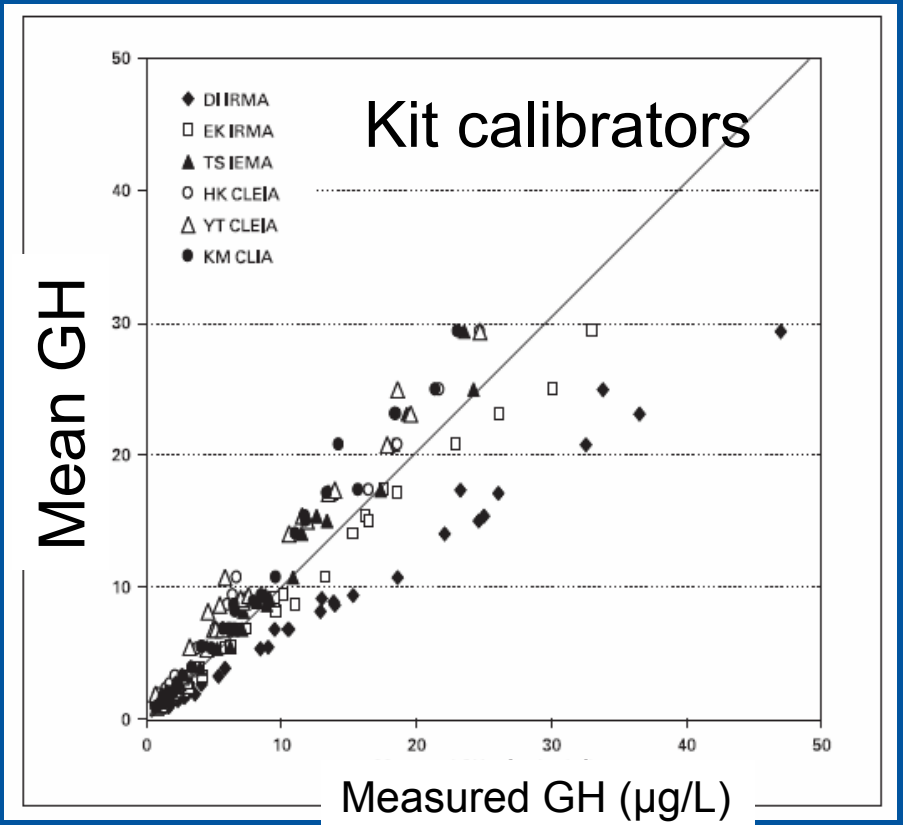
HORMONE RESEARCH

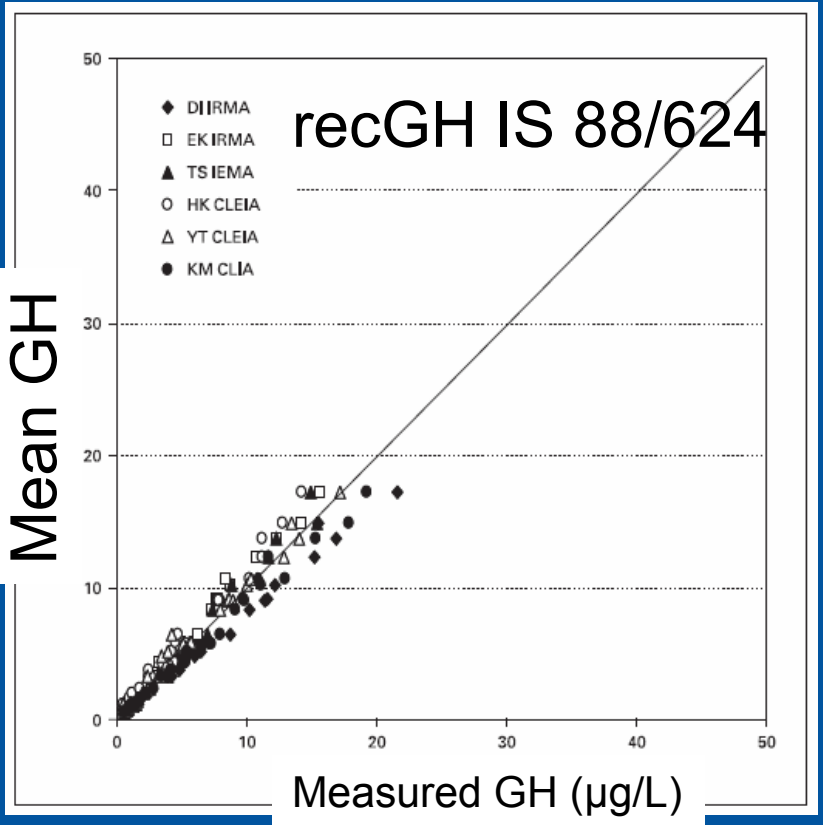
Horm Res 2005;64(suppl 2):6–11
DOI: 10.1159/000087746

2005

A Nationwide Attempt to Standardize Growth Hormone Assays

Toshiaki Tanaka[a, b] Katsuhiko Tachibana[a] Akira Shimatsu[a]





conclusies

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- Standaardisatie van immunoassays voor hormonen met meerdere (bioactieve) isovormen als bv GH, is niet mogelijk, tenzij de antistoffen selectief één isovorm herkennen

- Het gebruik van het harmonisatiemonster voor GH is een pragmatische oplossing om verschillen tussen methoden te reduceren.

- Internationaal overgaan op recombinant GH (98/574) maakt het noodzakelijk de cutoff waarden van ITT, GHRH/arg, glucagon testen opnieuw vast te stellen