

Measurements of GH and IGF-I: Implications for Diagnosis and Monitoring of GHD and Acromegaly

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GH & IGF-I Assay Consensus Paper

Clinical Chemistry 57:4
555–559 (2011)

Special Reports

Consensus Statement on the Standardization and Evaluation of Growth Hormone and Insulin-like Growth Factor Assays

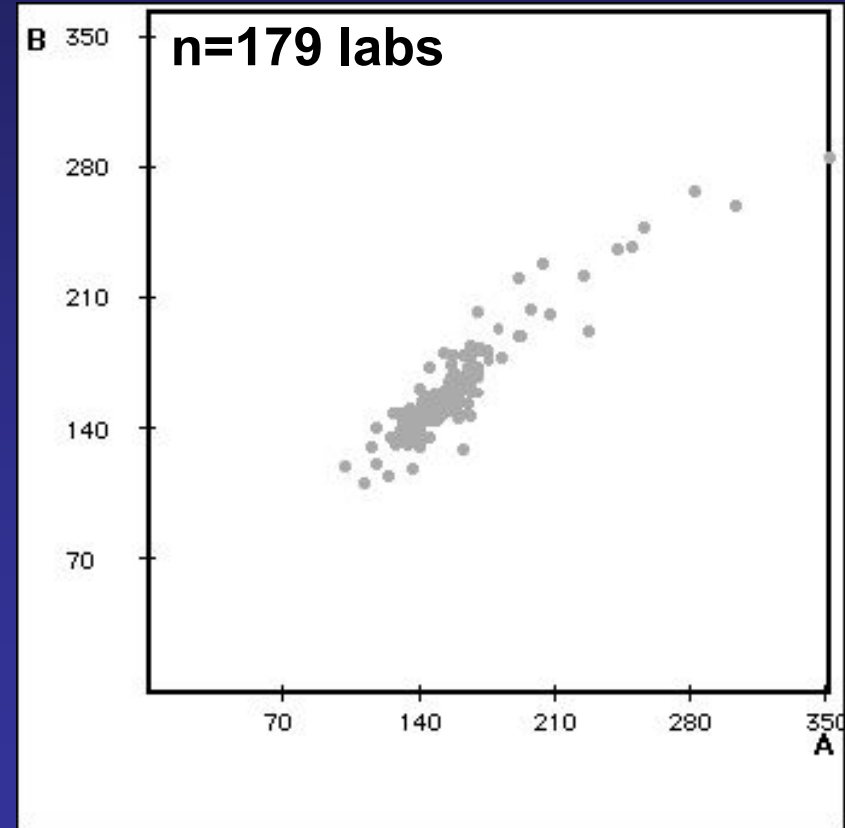
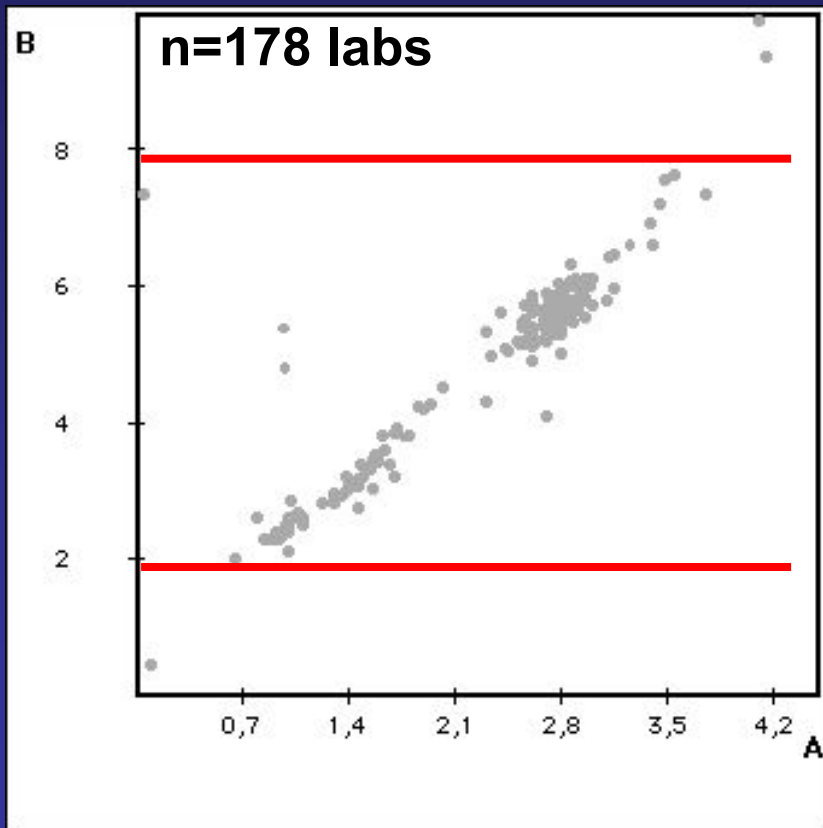
David R. Clemmons,^{1*} on behalf of the conference participants

Status 2010 / External quality assessment scheme for GH and IGF-I

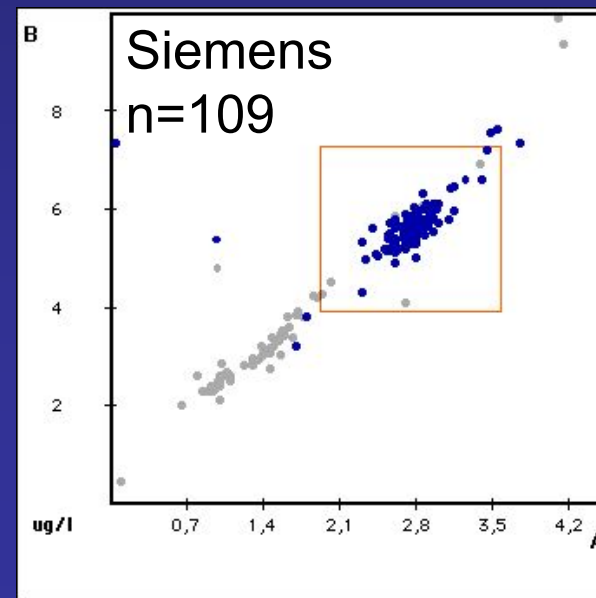
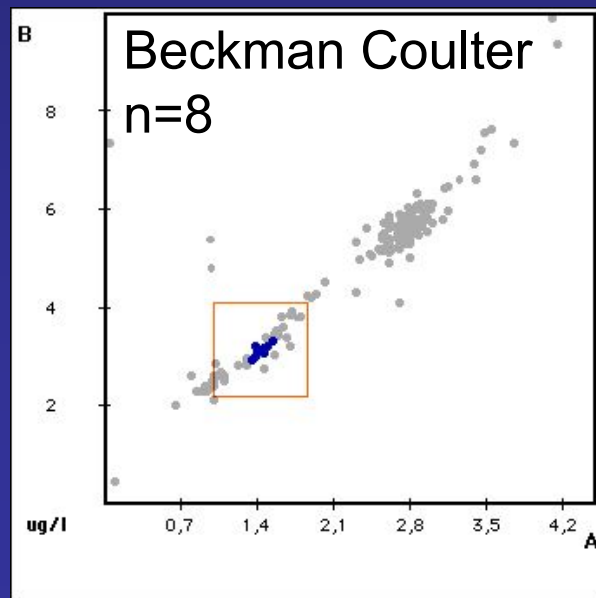
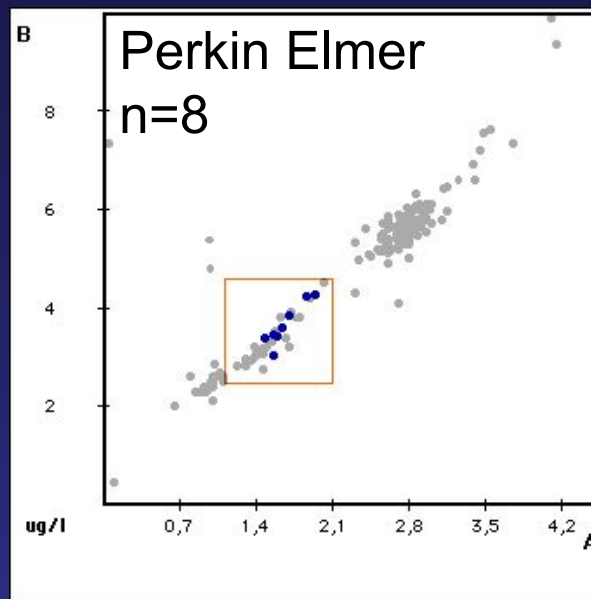
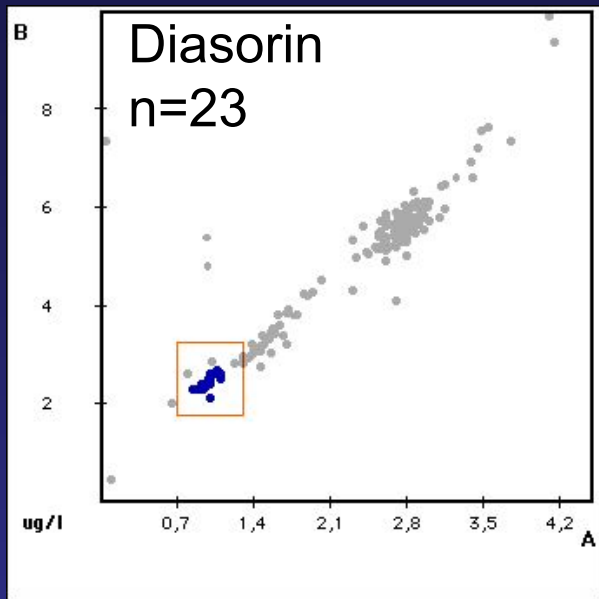
All labs analyse aliquots of the same samples (A and B).
Results centrally collected and plotted (each dot = 1 lab)

GH

IGF-I

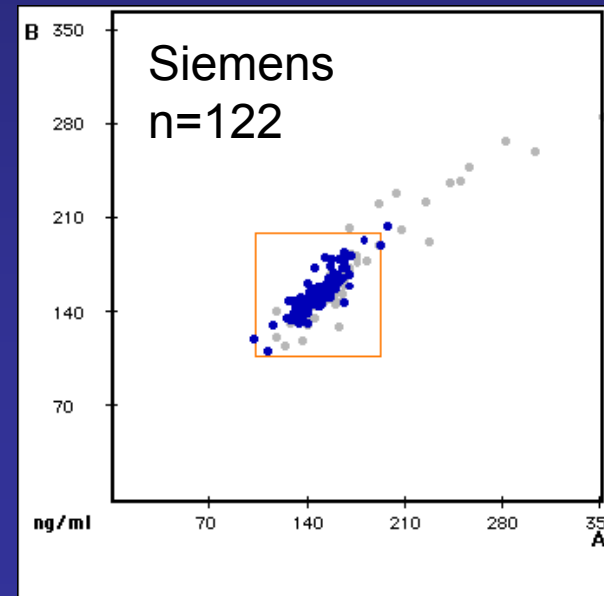
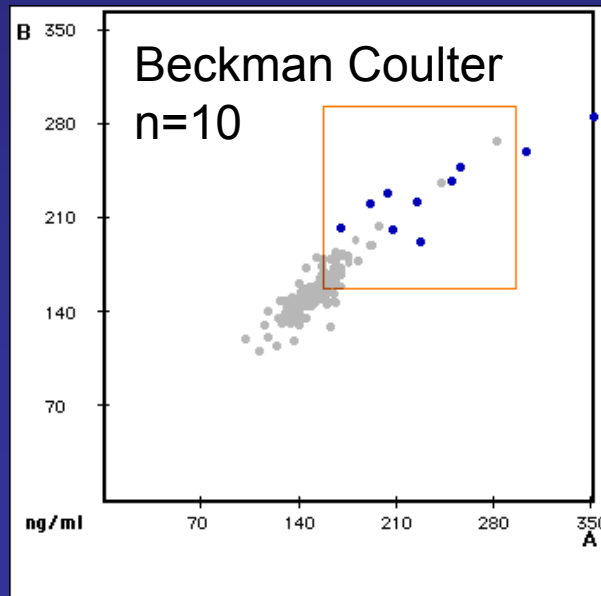
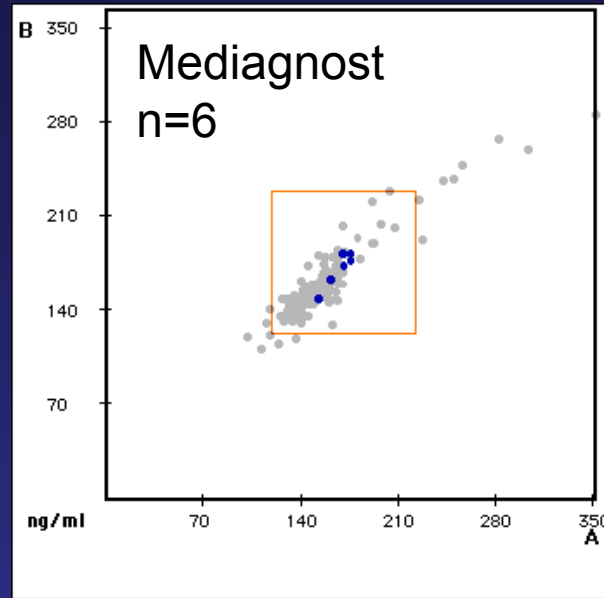
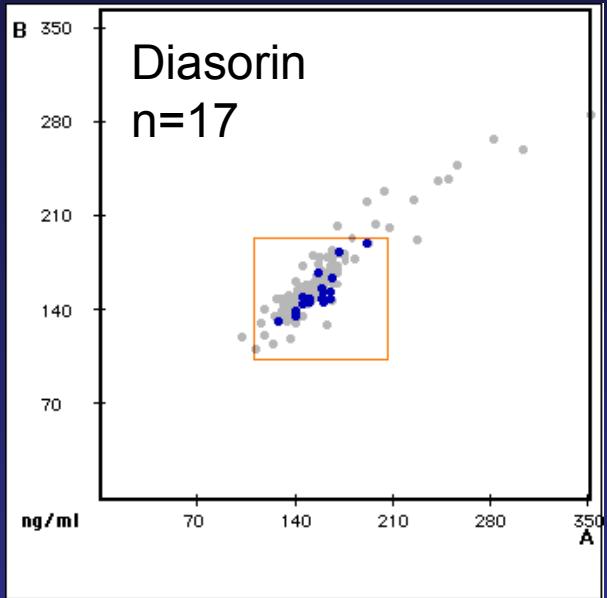


Status 1/2010 – EQAS for GH



Split by assay
method

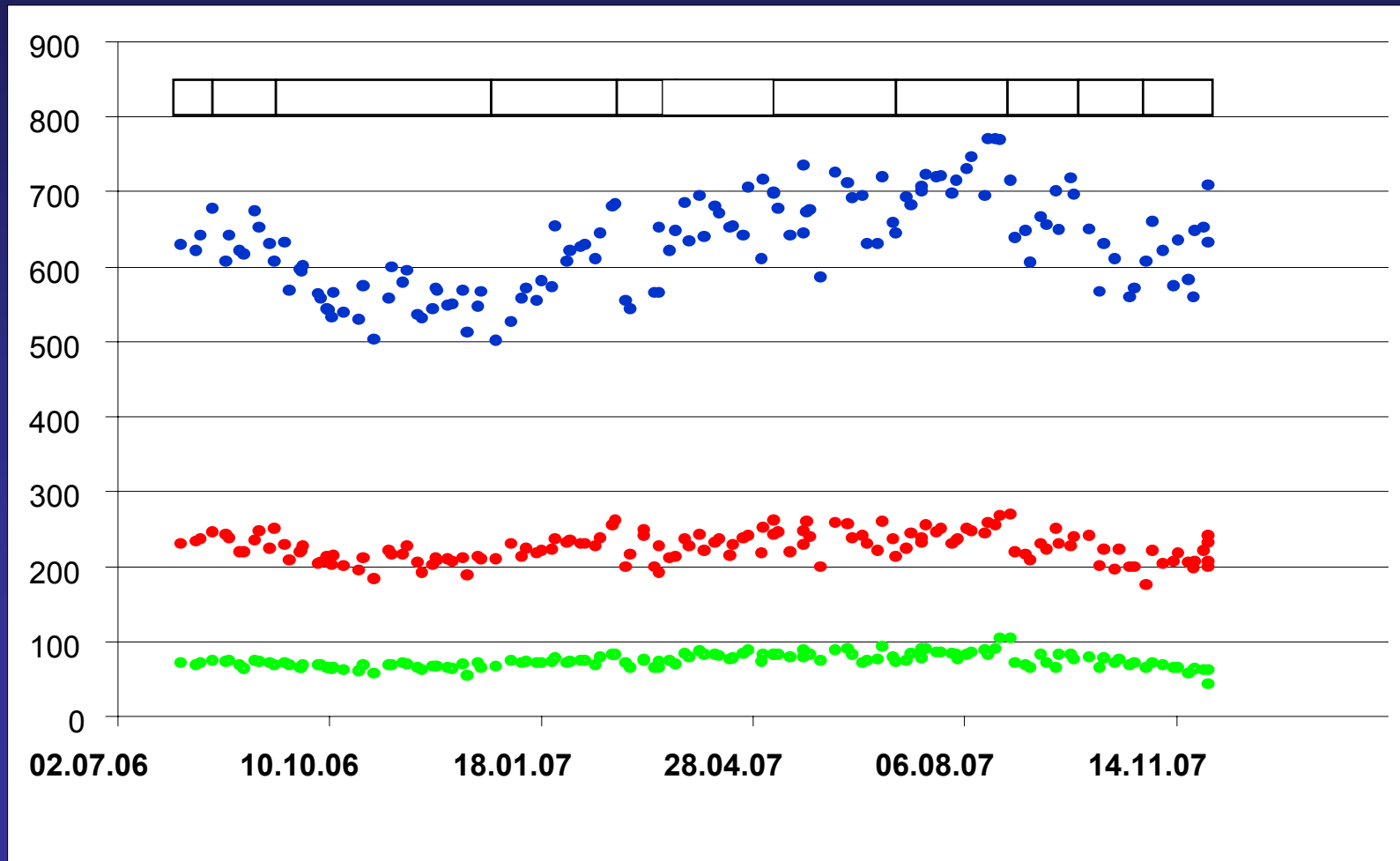
Status 1/2010 – EQUAS for IGF-I



Split by
assay method

Impact of between assay variability

DPC Immulite: Control pools 8/2006 – 11/2007



What do pediatric endocrinologists know about the GH assay used in their lab?

European Audit of Current Practice in Diagnosis and Treatment of Childhood Growth Hormone Deficiency

Anders Juul^a Sergio Bernasconi^b Peter E. Clayton^c Wieland Kiess^d
Sabine DeMuinck-Keizer Schrama^e for the Drugs and Therapeutics
Committee of the European Society for Paediatric Endocrinology (ESPE)

Horm Res
2002;58:233–241

- 235 participants (ESPE members)
- only 63% knew what GH assay they were using
- only 57% knew how their GH assay was calibrated
- “top” local cut off levels were 10 ng/ml and 20 mU/L – regardless which assay and calibrator was used!

What do adult endocrinologists know about the GH assay used in their lab?

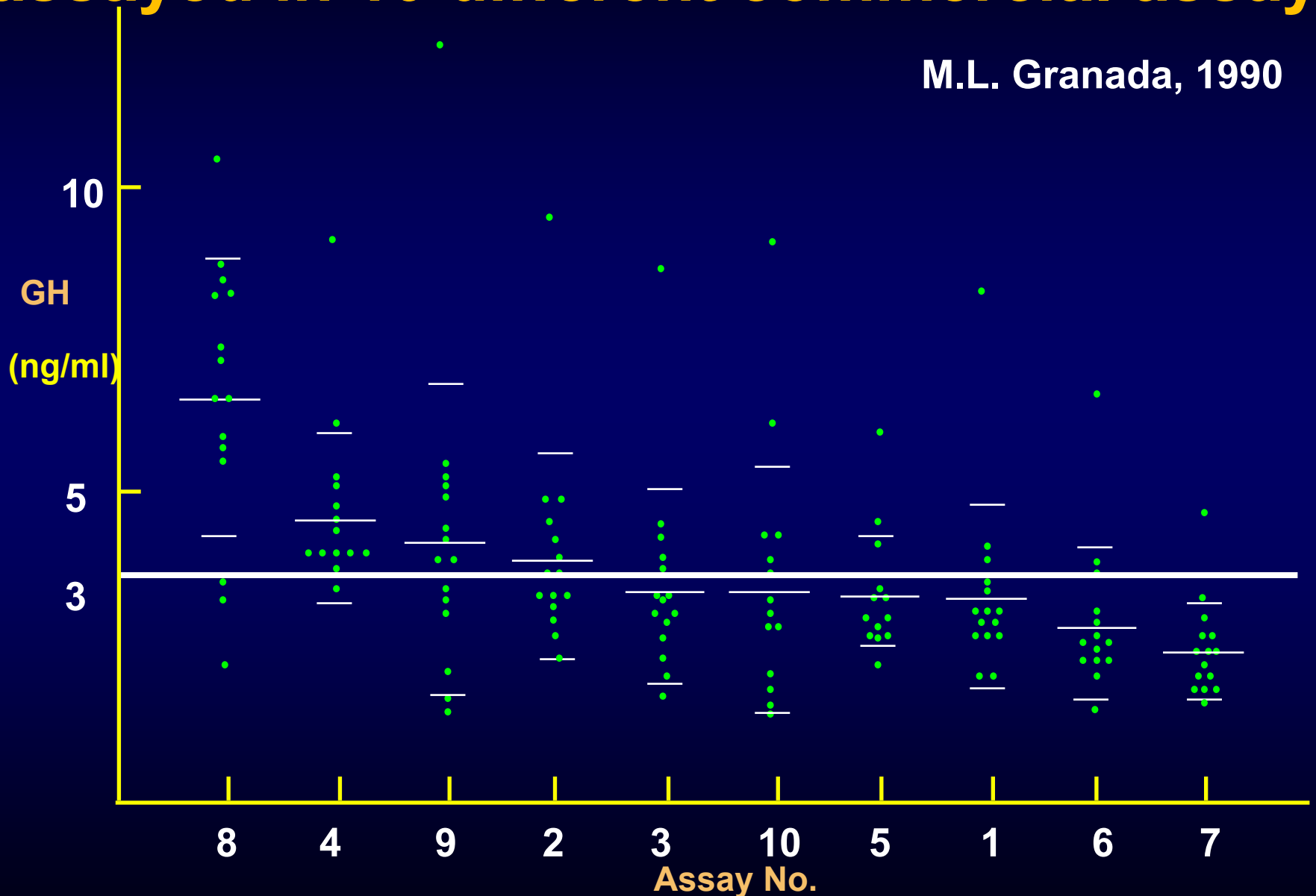
- **Nationwide multicenter study on biochemical markers of disease activity in acromegaly**
- **26 local centers with local labs participated**
- **independent survey among local physicians and local laboratories asking “which assays is used by local lab”**
- **Completely correct answer (manufacturer plus method) : 11%**
- **For 66% at least the company name was correct**

.. Why should they care ?

**Because GH results
from different labs
vary dramatically !**

Fifteen 24-h serum pools of short children assayed in 10 different commercial assays

M.L. Granada, 1990



NEQUAS: disparity of hGH results over time

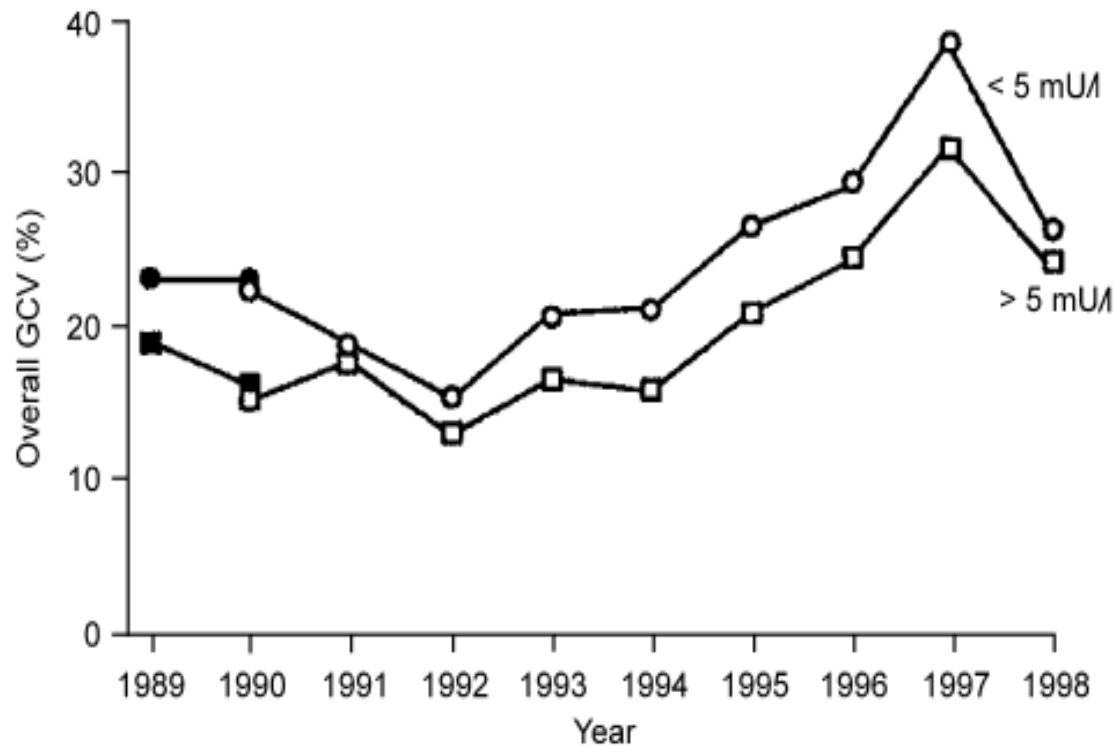


Fig. 1. Temporal trend of GH assay precision, represented as between laboratory agreement (geometric coefficient of variation, GCV) in the UK National External Quality Assessment Scheme. Inter-laboratory agreement has progressively deteriorated since 1994, coincident with the increasing use of monoclonal assays and commercial assay kits. This trend occurred despite increased automation of assay technology. See text for an explanation of potential reasons. Reproduced with permission from Seth et al, *Hormone Res* 1999;51(Suppl 1):13-19.

Preliminary conclusions

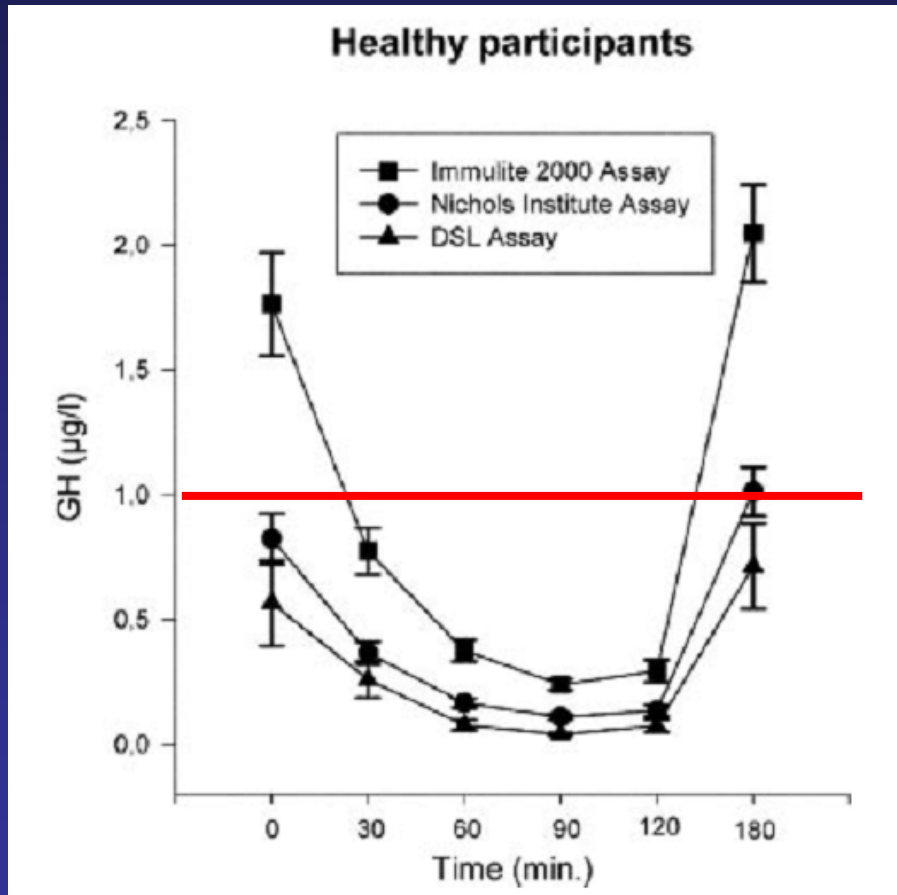
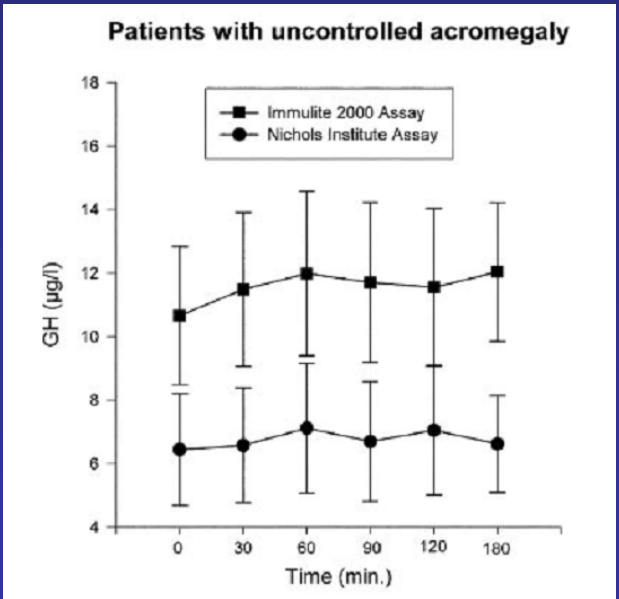
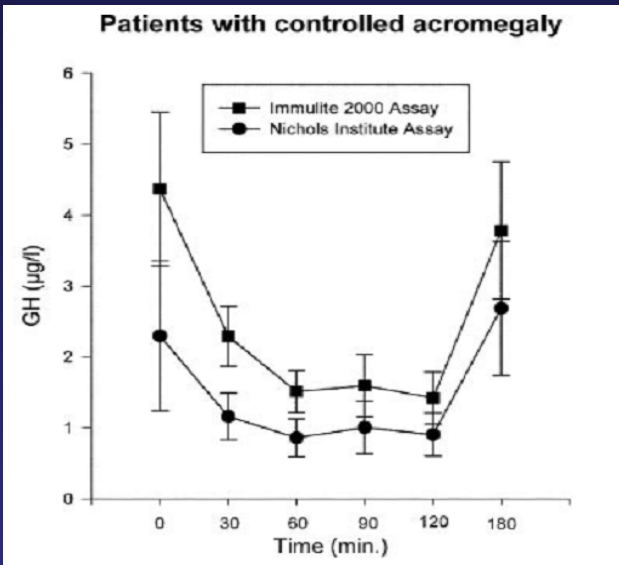
- **GH assays are inaccurate**
- **“Estimates” of circulating GH concentrations vary by more than 300%**
- **Presence or absence of active disease seems to depend on which assay is chosen by the lab**
- **There was absolutely no improvement over the last 20 years**
- **It is a waste of time to discuss about cut-off levels for clinical guidelines**

Why is it so difficult to measure GH?

All GH assays in routine analytics are immunoassays:

- **Impact of antibody specificity (different antibodies recognize different epitopes)**
 - **pituitary GH consists of several isoforms**
 - **GH binding protein potentially covers epitopes**
- **Impact of standard preparation**
 - **pituitary and recombinant GH standards exist**
 - **different conversion factors between the two units mU/L and ug/L are in use**

Clinical Relevance of Assay-Problem: oGTT



Problems with IGF-I assays

Clemmons DR, Horm Res 2001; 55 (suppl 2): 73-79

1. Analytical problems

- Calibration (standardization)
- Separating IGF-I from binding proteins
- ***Intra- and inter-assay variability***

2. Problems with interpretation

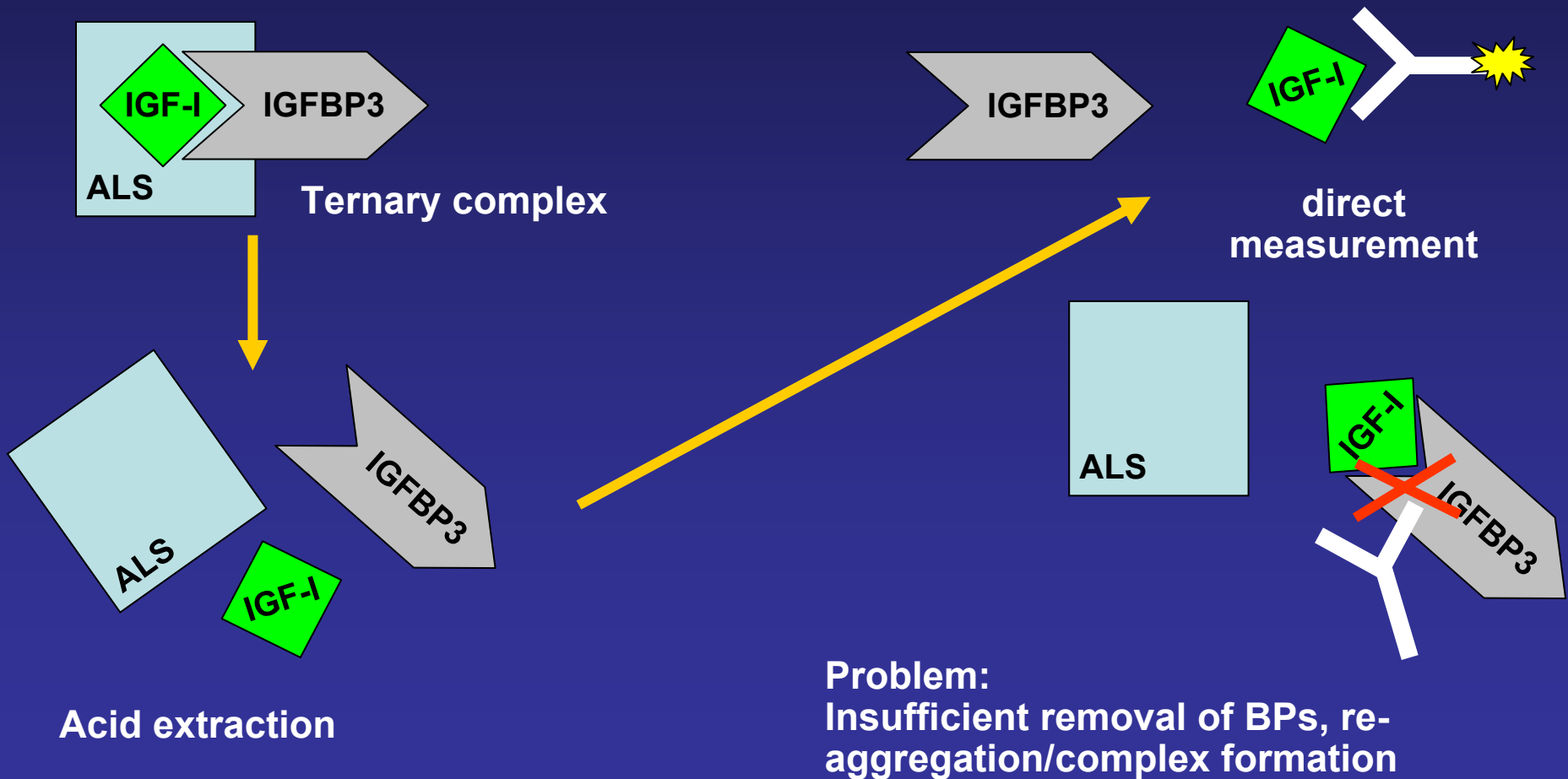
- Sufficient high-quality normative data
- Evaluation under clinical conditions
- ***Biological variability***

Why different assays give different results

- **Issues with antibody specificity and binding proteins**
- **Issues with standard preparations and calibration**
- **Issues with assay precision and lot-to-lot stability of reagents**

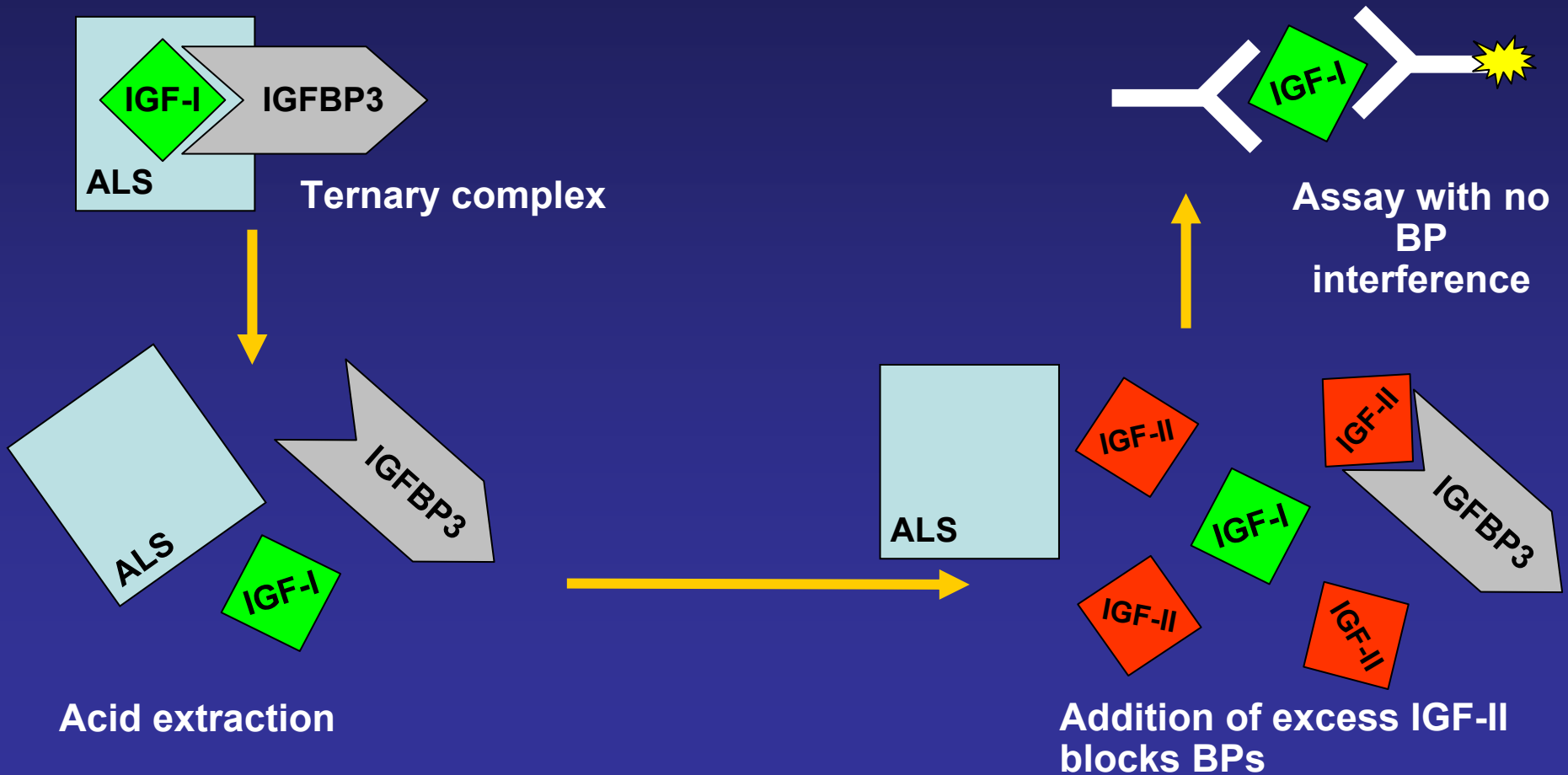
Issues with antibody specificity and BPs

„Direct“ IGF-I-Assays



Issues with antibody specificity and BPs

„Gold standard“: IGF-II-Displacement



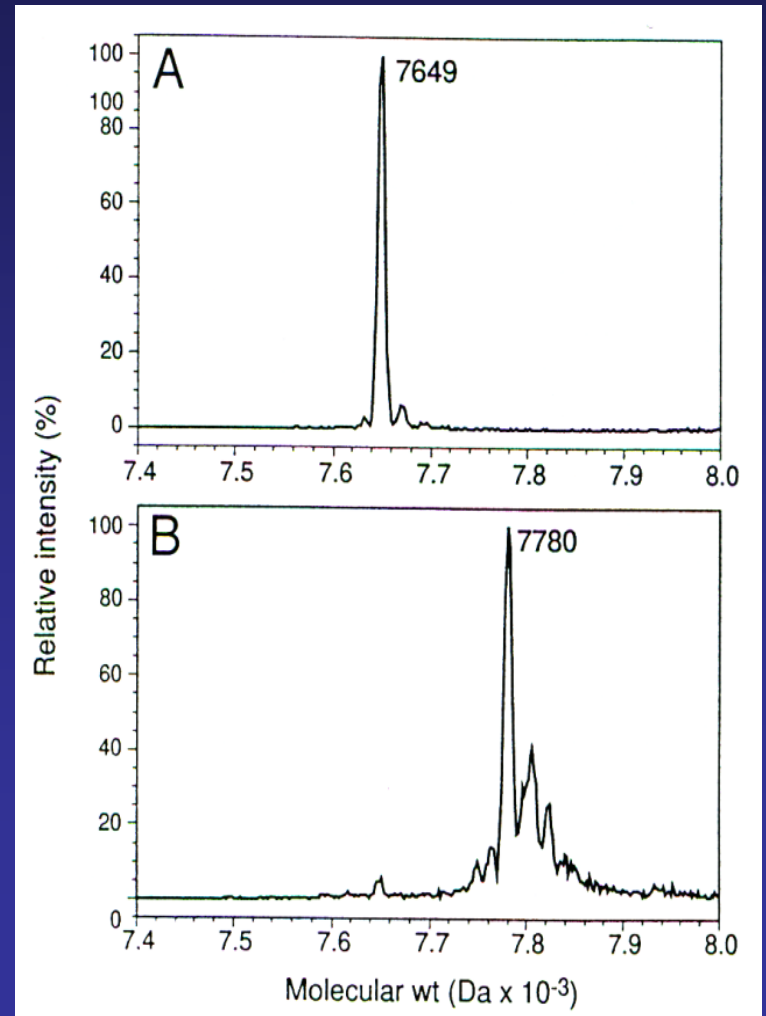
IGF-I assays: Calibration

Quarmby V et al., JCE&M 1998; 83, 1211-1216

Genentech rhIGF-I:
MW 7649

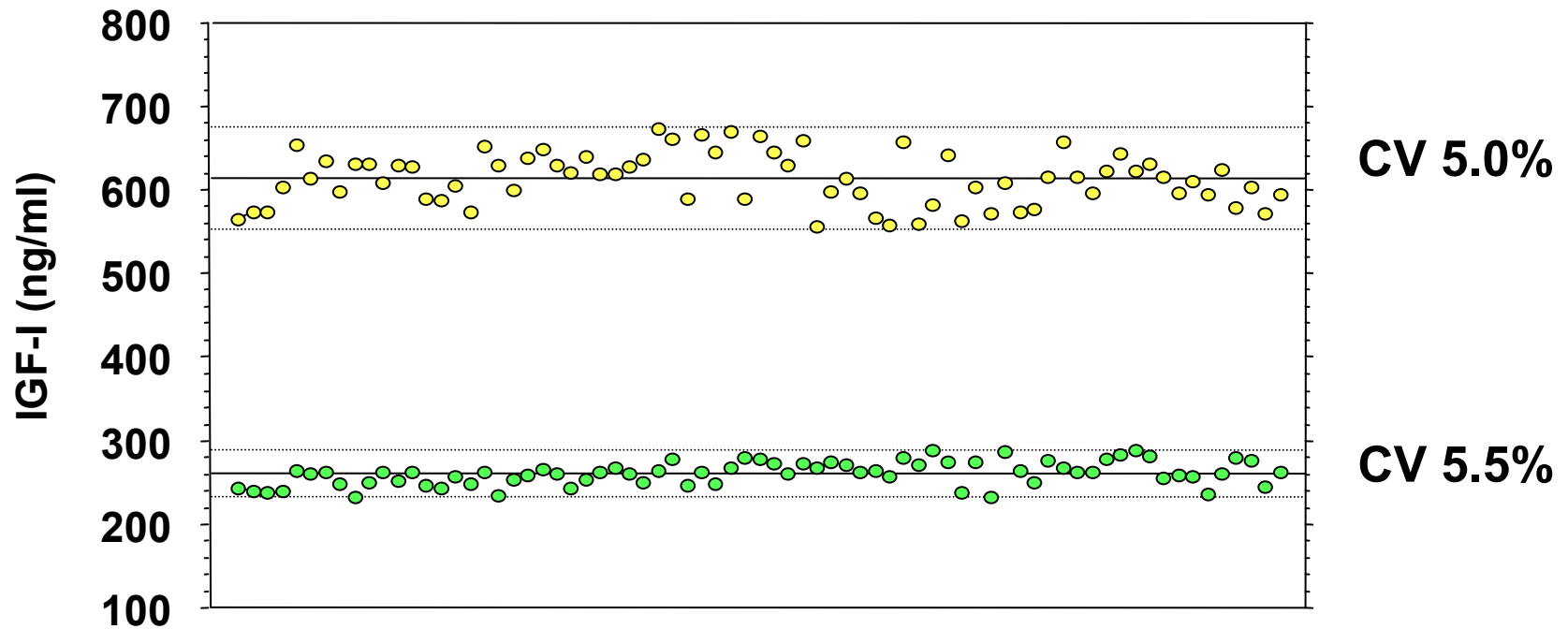
WHO IRP 87/518:
rhMet¹-IGF-I, low purity (44%), MW
7780

Assigned IGF-I protein content
relatively too high



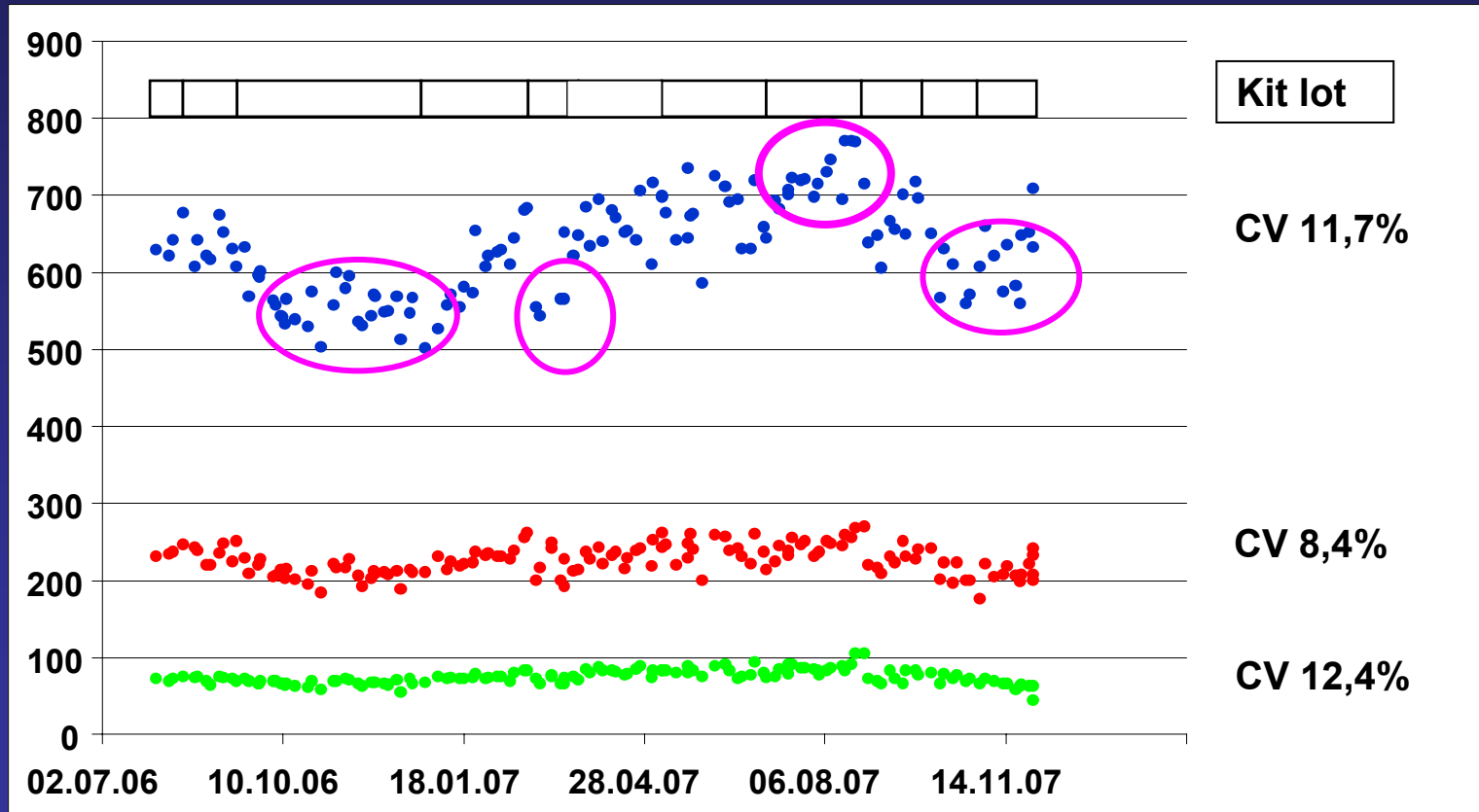
Impact of between assay variability

Nichols Advantage IGF-I: Control pools 4/2001 – 4/2002



Impact of between assay variability

DPC Immulite: Control pools 8/2006 – 11/2007



Why is this so relevant?

Harmonization and comparability of GH and IGF-I assays are required to provide transparent and useful guidelines for management of GH/IGF-I axis disorders

If we get discrepant numbers, we can't use the same recommendations!

Problem reflected in guidelines

In a child with clinical criteria for GHD, a peak GH concentration below 10 $\mu\text{g/L}$ has traditionally been used to support the diagnosis. This value needs to be revised when using newer monoclonal-based assays and recombinant hGH reference preparations. There exists a continuum of GH

Childhood GHD Eilat Consensus statement (published JCE&M 85(11), 2000)

Biochemical assessment of the patient with acromegaly

Biochemical evaluation of the patient with possible acromegaly status includes measurements of serum concentrations of IGF-I and GH and study of the neurosecretory regulation of GH secretion through dynamic testing (1). The analysis of serum GH and IGF-I concentrations is limited by the lack of standardization and diverse technical problems with current and previous assays.

Acromegaly Feldafing Consensus statement (published JCE&M 89(7), 2004)

Recent activities to achieve GH and IGF assay harmonization

Attempts to improve the situation

International collaborative on GH assays

European Journal of Endocrinology (2006) 155 1–2

ISSN 0804-4643

CONSENSUS STATEMENT

Consensus statement on the standardisation of GH assays

“As a first step we recommend to only report GH assay results in $\mu\text{g/l}$ (ng/ml) of the recombinant calibrator IS 98/574”

Attempts to improve the situation

October 2009 Keswick, VA

Consensus Workshop on “GH and IGF-I Assays: present and future”

Organized by Growth Hormone Research Society (GRS) in collaboration with

- International Federation for Clinical Chemistry and Laboratory Medicine (IFCC)
- International Society for IGF Research
- Pituitary Society

Participants: Clinical and laboratory experts, health authorities (FDA, EMEA), quality assurance organizations (UKNEQAS, CAP...), metrological institutions (IRMM...) etc.

Problems identified...

...for GH and IGF-I assays

1. **Use of different standard preparations and reporting units**
2. **Analyte heterogeneity and antibody specificity**
3. **Binding protein interference**
4. **Preanalytics and matrix interference**
5. **Interpretation and normative data**
6. **QC procedures**

Blue background: GH related

Green background: IGF-I related

Standard preparations: GH

Code	source	mg/amp	U/mg
66/217	pit	0.175	2.0
80/505	pit	1.7	2.6
88/624	rhGH	2.0	3.0
98/574	rhGH	2.0	3.0

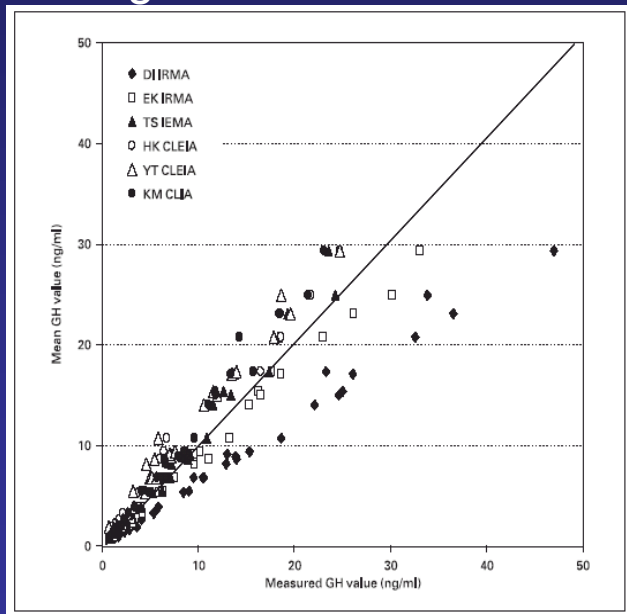
Which preparation to be used? How to convert units to mass? How to report results?

Standard preparations: GH

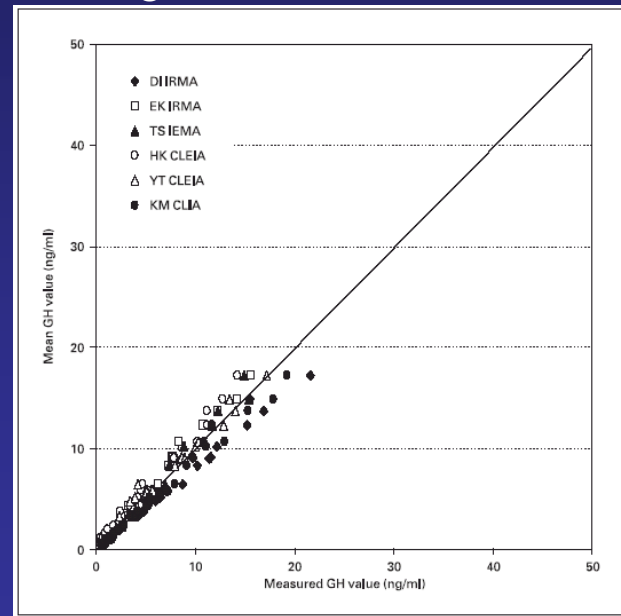
Use of only one calibrator reduces assay variability

GH measured in 60 samples by the 6 most frequently used assays

Using Kit-calibrators



Using same rec. calibrator



Reduction in mean variability from 35% to 18%

Reporting GH results

Conversion units/ μg in papers on acromegaly published by a leading journal:

- Orme et al., JCE&M 1998 (Mortality and cancer in acromegaly): factor used 2.0, $5 \text{ mU/l} = 2.5 \mu\text{g/l}$
- Kaltsas et al., JCE&M 2001 (Predictors of outcome of surgical treatment): factor used 2.5, $5 \text{ mU/l} = 2.0 \mu\text{g/l}$
- Baldelli et al., JCE&M 2000 (2 year follow-upo slow release lanreotide): factor used 3.0, $5 \text{ mU/l} = 1.6 \mu\text{g/l}$
- Cozzi et al., JCE&M 2003 (4 year octreotide vs. short term results): factor used 2.5: $5 \text{ mU/l} = 2.0 \mu\text{g/l}$, in the same paper for own results factor 2.6: $5\text{mU/l} = 1.92 \mu\text{g/l}$

Standard preparations: GH

Workshop recommendations:

GH assays should all be calibrated with 2nd International Standard for somatotropin (recombinant DNA derived hGH (IS 98/574))

GH results should be expressed in mass units ($\mu\text{g/L}$) of IS 98/574

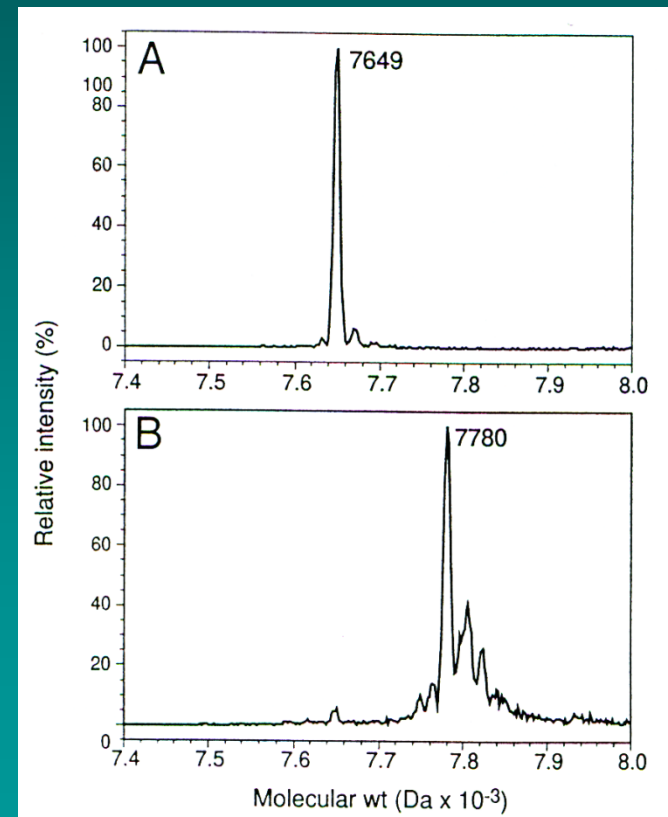
The European Journal of Endocrinology starting from January 1 2007 will publish papers on GH data only if expressed in mass units of IS 98 574.

Standard preparations: IGF-I

Old IGF-I standard is impure and concentration is incorrectly assigned!

“theoretical” gold standard
e.g. Genentech rhIGF-I:
MW 7649

“real life” WHO IRP 87/518:
rhMet¹-IGF-I, impure (44%),
MW 7780



Quarmby V et al., JCE&M 1998; 83, 1211-1216

Standard preparations: IGF-I

Workshop recommendations:

IGF-I assays should be recalibrated with the new IS 02/254 WHO reference standard

IS 02/254 is >97% pure recombinant standard, well characterized by the National Institute of Biological Standards and Controls (NIBSC) in an international multicenter study and bioactive

Analyte heterogeneity and antibody specificity: GH

<u>GH isoforms in serum</u>	(%)
22 kDa monomer total	48
20 kDa monomer total	9
Acidic GH (desamido- & acyl-GH)	5
22 kDa non-covalent dimers	14
22 kDa disulphide dimers	6
20 kDa non-covalent dimers	5
22 kDa non-covalent oligomer	7
22 kDa disulphide oligomer	3
Others	3

Analyte heterogeneity and antibody specificity: GH

- **Assays differ in the epitope specificity of the antibodies employed in the assay**
- **Different antibodies bind a different subset of molecular isoforms of GH**
- **GH assays therefore do not all measure the same analyte or “measurand”!**

Analyte heterogeneity and antibody specificity: GH

Workshop recommendations:

GH assays should be specific for 22 kD GH (most abundant isoform)

Manufacturers of GH assays should clearly specify assay cross-reactivity with 20K GH, placental GH and GH analogs (eg. Pegvisomant)

Binding protein interference - GH

Workshop recommendations:

GH assays must specify the degree of interference by GHBP within its physiological range

Samples should be incubated with GHBP for >12h to allow GH/GHBP complex formation prior to measurement

Binding protein interference - IGF-I

- **IGF-I assay performance is affected by the presence of IGFBPs in the sample**
- **IGFBPs interfere with IGF-I/antibody interaction, thereby reducing detection resulting in falsely low values**
- **Gold standard for removal of IGFBP interference is gel chromatography performed at low pH, but routine methods need easier techniques**

Binding protein interference - IGF-I

Workshop recommendations:

The method for prevention of IGFBP interference should be stated for each assay

Validation should include serum from patients with GH disorders, Type 1 DM, CRF and hepatic disease

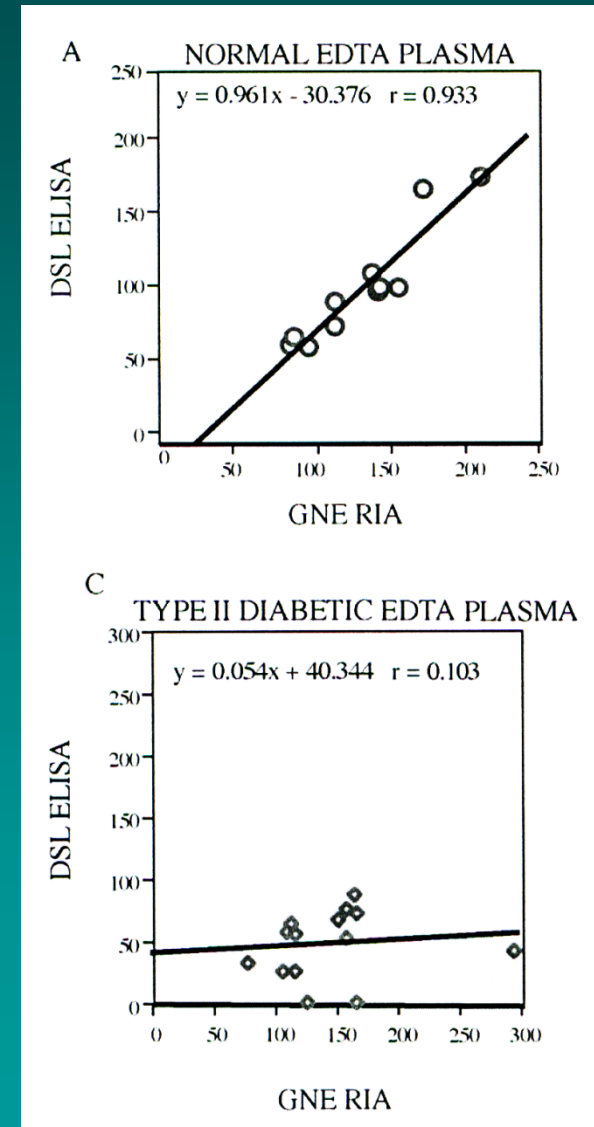
IGF-I assays: Difficulties in specific clinical conditions

Chestnut RE et al.,

J Immunol Methods 2002; 259: 11-24

Comparison of IGF-I-assays in samples from healthy volunteers and diabetic patients

“direct assays”:
Acceptable correlation in
healthy subjects, no
correlation in diabetics!



Binding protein interference - IGF-I

Workshop recommendations:

Assays must demonstrate that interference by IGFBPs is substantially removed

To validate this, samples must be spiked with IGFBP-2 and IGFBP-3 concentrations up to twice the highest levels likely to be encountered in clinical samples

Preanalytics / matrix: GH & IGF-1

Workshop recommendations:

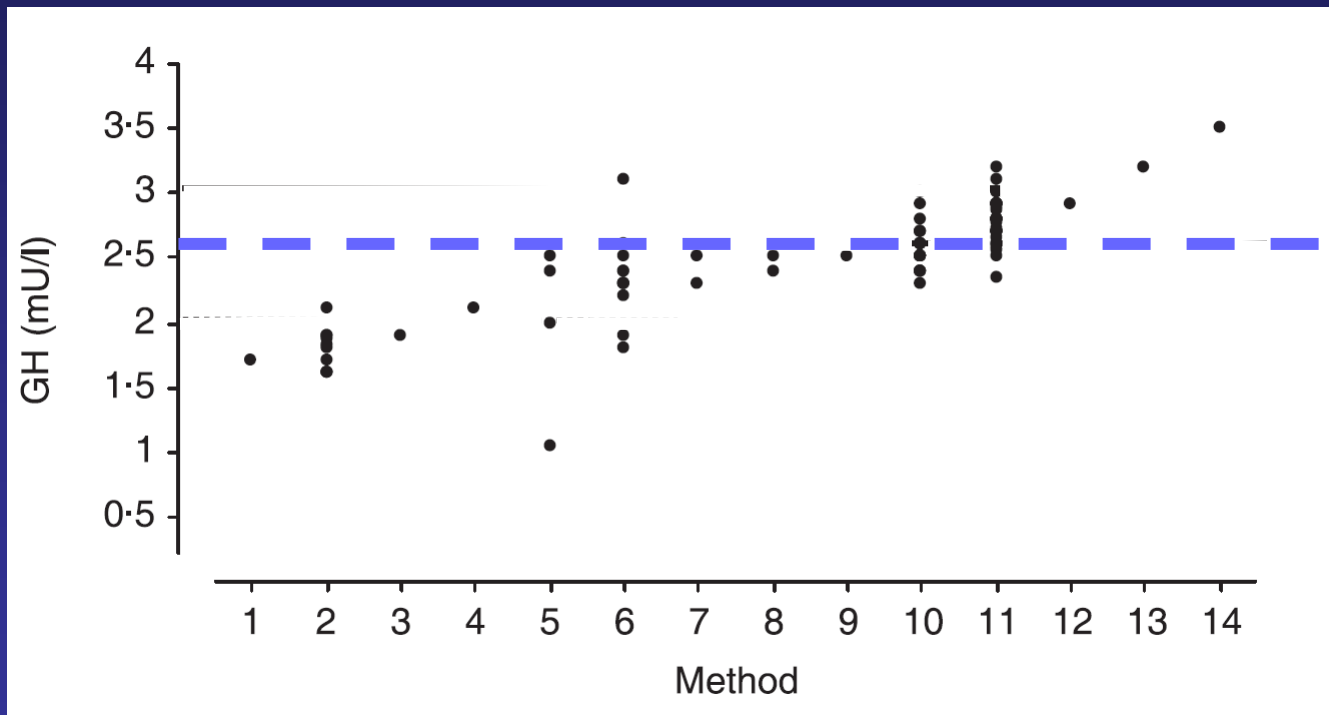
Timely separation from serum and blood cells recommended

Serum is recommended, use of anticoagulants requires separate validation (e.g. EDTA plasma)

Matrix of calibrators must mimic properties of samples (e.g. BPs!)

Normative data / interpretation: GH

One OGTT nadir sample (acromegalic patient)
measured by 14 assays in several labs



Normative data / interpretation: GH

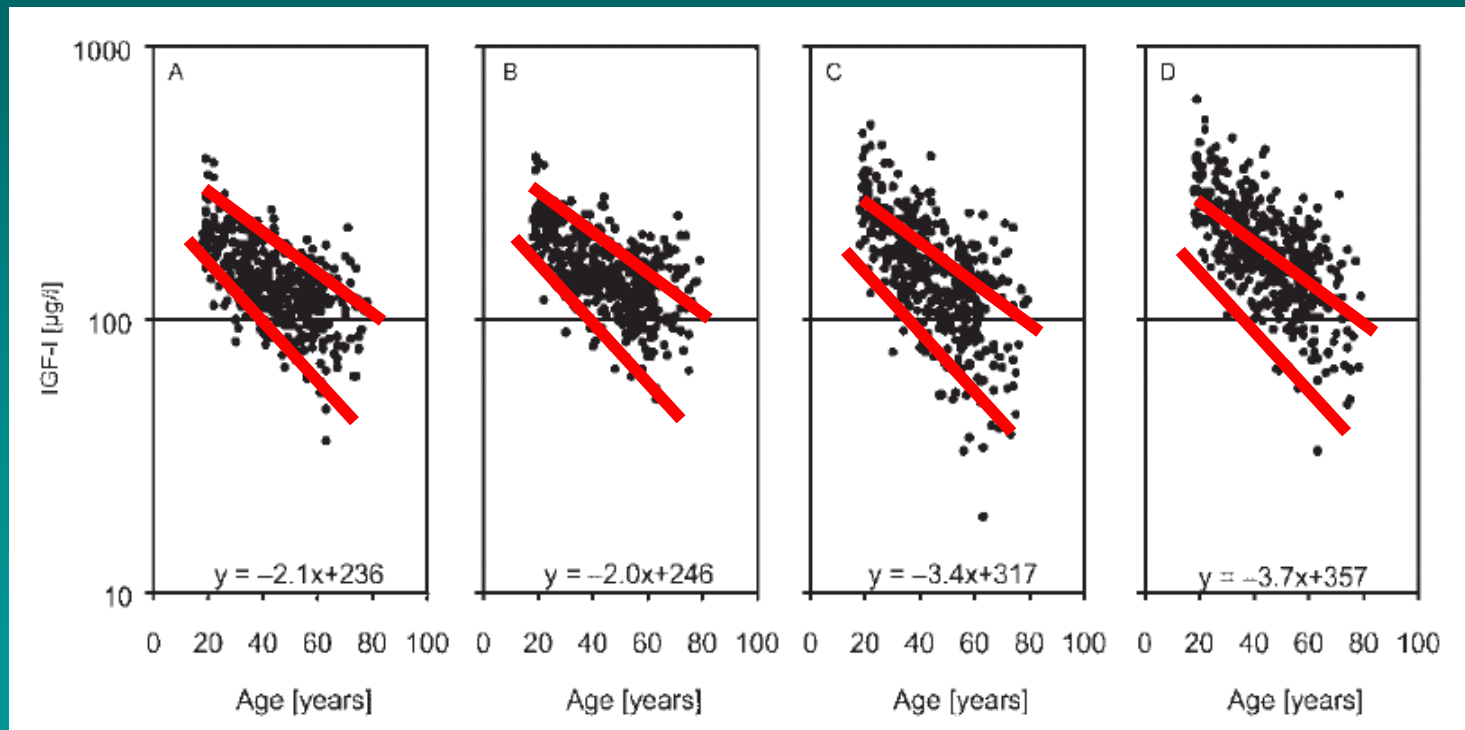
Workshop recommendations:

GH assay specific cut offs are required for interpretation of stimulation or suppression tests

For diagnosis of acromegaly (OGTT), GH assays should allow quantification of GH levels with CV of <20% at $\leq 0.05 \mu\text{g/L}$

Normative data / interpretation: IGF-I

700 samples from healthy adults measured by 4 IGF-I assays



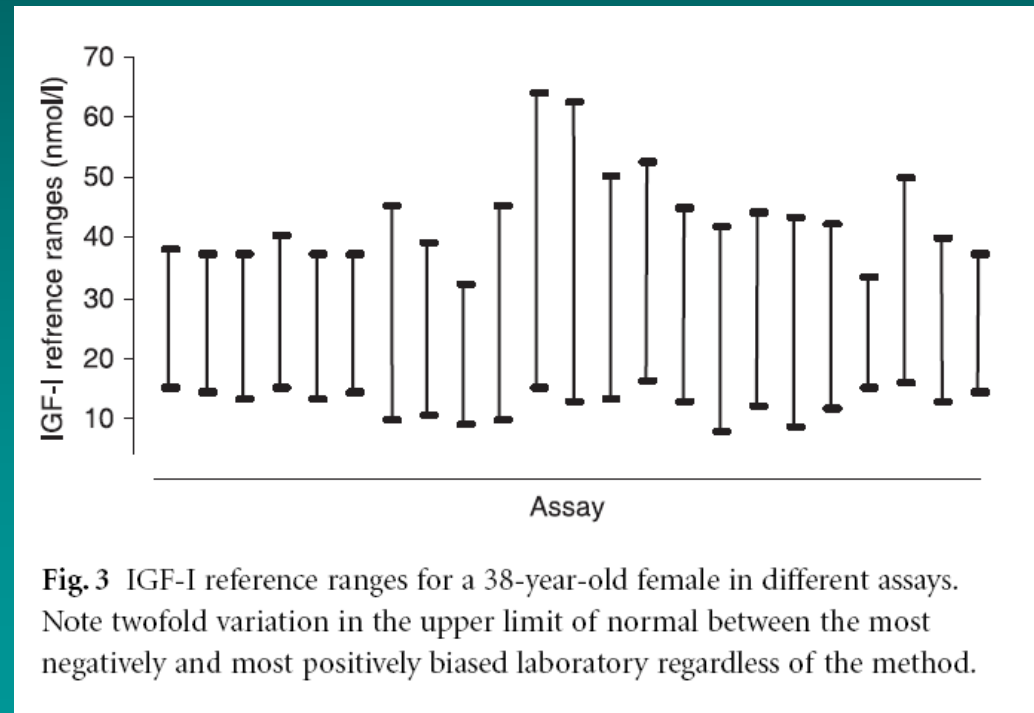
Normative data / interpretation: IGF-I

Quality of reference ranges reported by laboratories?

Aliquots of 1 sample sent to 23 labs

23 labs reported use of 6 different IGF-I-Assays

- but provided 15 different reference ranges



Normative data / interpretation: IGF-I

Workshop recommendations:

Normative data must be method specific and based on random selection from background population

Stratification of age groups based on statistical analysis of published data (to define subjects needed within each age-range)

Narrow age ranges required in childhood and adolescence (incl. Tanner stages)

Normative data / interpretation: IGF-I

Workshop recommendations:

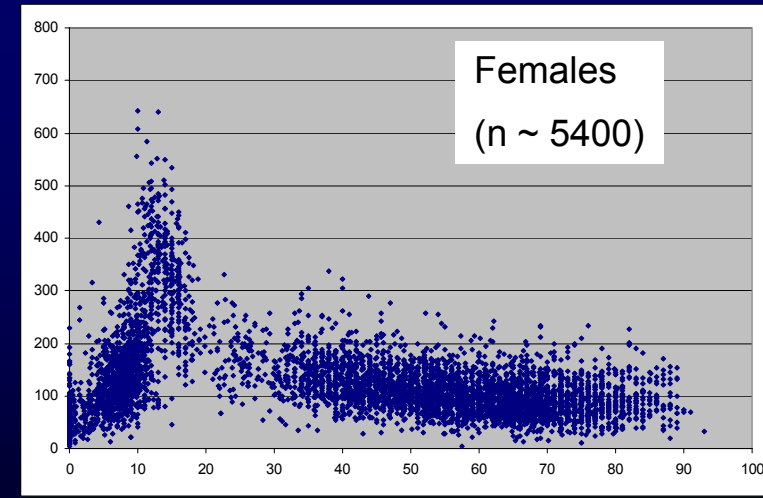
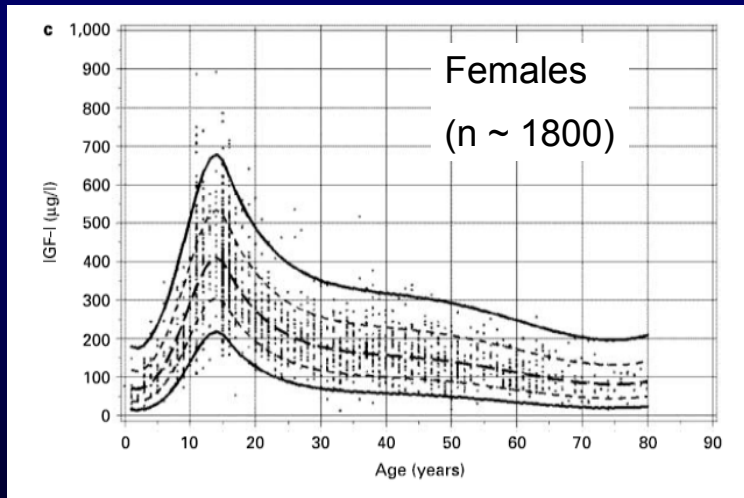
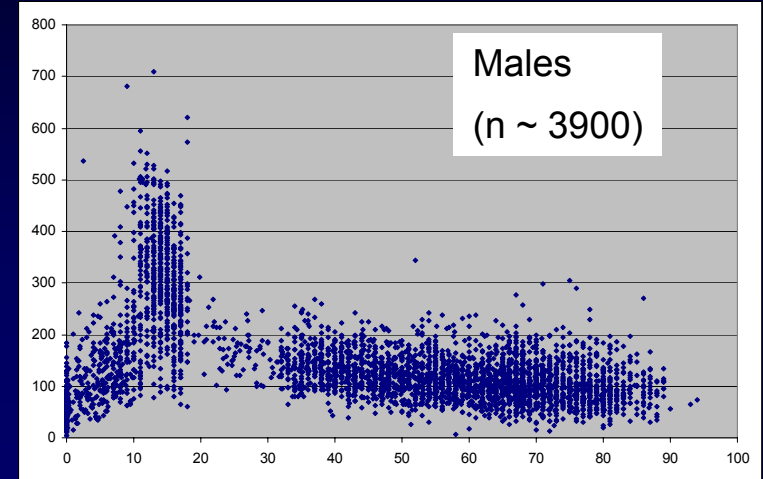
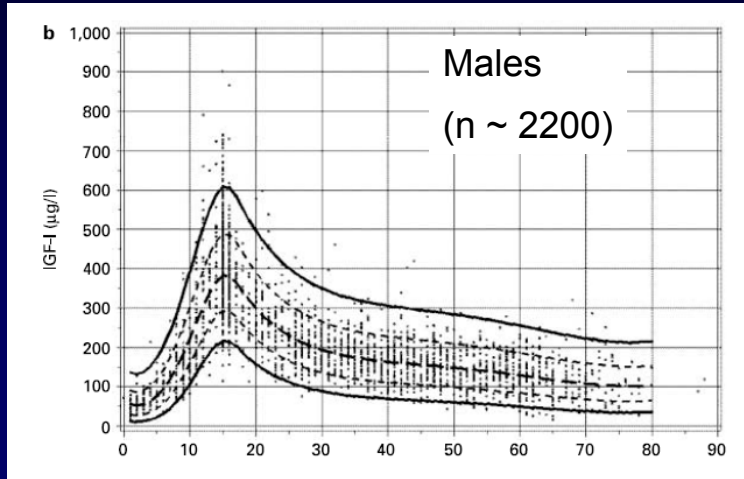
Gender specific levels for IGF-I are required within the age-range of 6 to 18 years

In adults gender and ethnicity minor confounders

Across BMI ~22-37 kg/m² little change in IGF-I.

Above / below significantly lower IGF-I values observed – cautious interpretation

Normative data for IGF-I-Assays



Nichols Advantage
Brabant et al., Horm Res 2003

Evaluation IDS iSys 9/2010
Unpublished

Quality control: GH & IGF-I

Workshop recommendations:

Internal quality control pools should be used independent of those provided by the manufacturer

Laboratories should participate in accredited proficiency testing/external quality control assessment schemes (PT/EQA)

Summary

- **GH and IGF assay results still vary by more than 200% between methods**
- **Analyte heterogeneity, antibody specificity, binding protein interference and differences in assay standardization are the main reasons for the discrepancies**

Conclusion

- **Manufacturers must adopt the proposed steps in terms of standardisation and validation**
- **Clinicians must be aware of the assay issue and the consequences for interpretation**
- **You need to know the assays your lab uses to analyze your patients` samples**
- **The implementation of new better assays will affect the cut-off thresholds**

Acknowledgements

GRS workshop POC

David Clemmons (Chair)

Martin Bidlingmaier

Jens Sandahl Christiansen

Gudmundur Johannsson

John Kopchick

Michael Thorner

International collaborative on
GH assays

Gilbert Wieringa

Peter Trainer

Cathie Sturgeon

Take-home message:

**In both
Immunoassays or Fishing
you have to get your tools
right!**

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