



# **IGF-I RIA**

**Radioimmunoassay  
for the Quantitative Determination of  
Insulin-like Growth Factor-I  
(IGFBP blocked)**

**For Research Use Only**

**Catalog Number: 022-IGF-R21**  
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## FEATURES

- High Specificity for IGF-I
- Correct measurement of IGF-I in non-extracted samples
- No physical separation of IGF-I from IGF-binding proteins required
- Elimination of interference by IGF-binding proteins through excess IGF-II
- 100 % recovery of IGF-I leads to correct absolute values
- Precise measurement of very low IGF-I levels
- Small sample volume requirement, thus ideal for young patients
- Convenient method for correct measurement of IGF-I in samples containing low IGF-I and high IGFBP (e.g. cell culture media)
- More reliable performance by means of coloured solutions

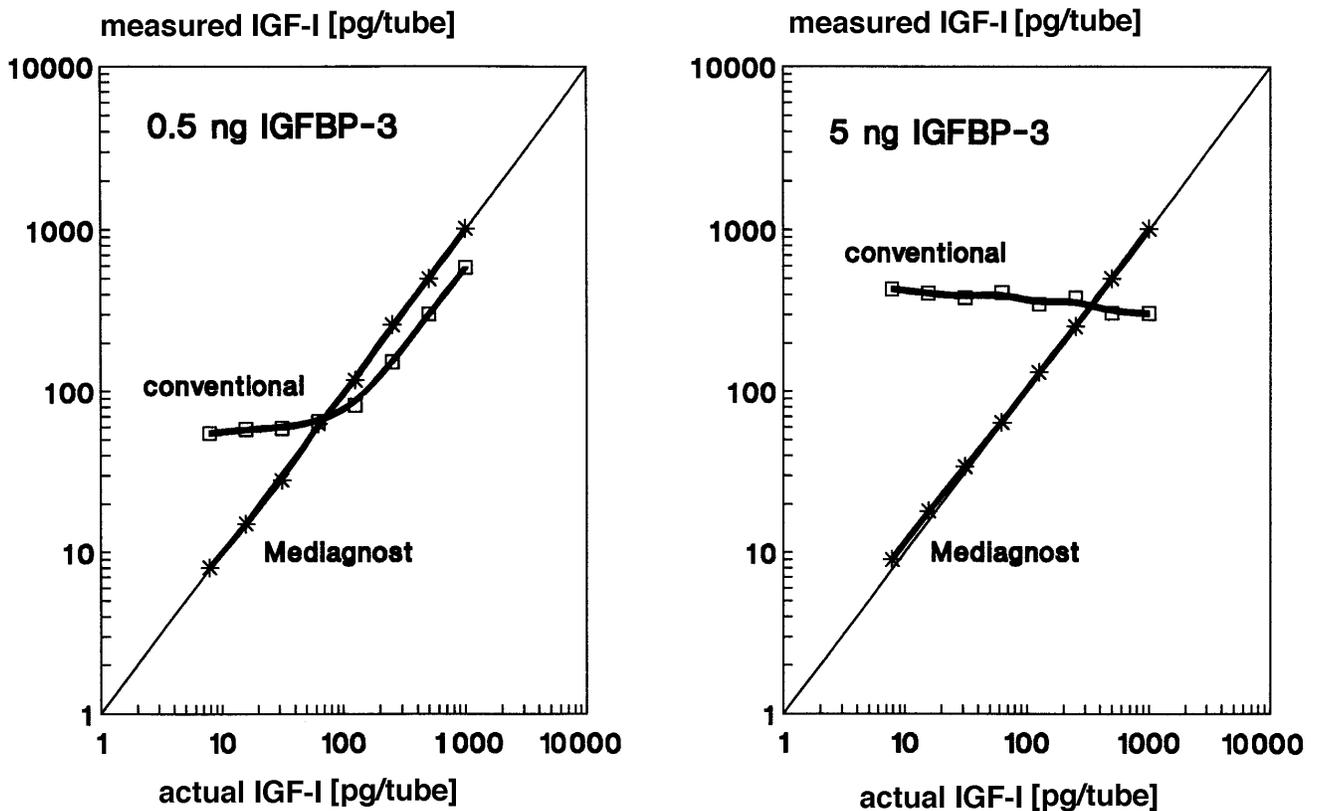
## BACKGROUND

Insulin-like growth factors (IGF) I and II play a pivotal role in regulating the proliferation, differentiation and specific functions of many cell types (1-3). IGF-I is identical with Somatomedin C (Sm-C) (4) and has a molecular weight of 7649 daltons (5). Its major regulators are growth hormone (GH) and nutrition (6), although its production in specific tissues is affected by a multitude of tropic hormones and other peptide growth factors. In contrast to many other peptide hormones, IGFs are avidly bound to specific binding proteins (IGFBP). The seven classes of IGFBPs which are known at present (7,8,22) either bind IGF-I and IGF-II with similar affinities or show a preference for IGF-II (9,10).

A major problem of IGF-I measurement results from the interference of IGFBPs in the assay. Direct determinations in untreated serum samples (11) give false values because of the extremely slow dissociation of the IGF-I/IGFBP-3 complexes during the assay incubation. Depending on the ratio IGF-I to IGFBP the following errors may occur (see also Figure 1):

- in samples with low IGF-I concentration, IGFBP-complexation will take place predominantly with the IGF-I-tracer, thus leading to false-high results in a competitive RIA. Effect: Overestimation of low IGF-I levels.

- in samples with high IGF-I concentration, unmarked IGF-I from the sample will be predominantly complexated by IGFBPs and therefore withdrawn from measurement. Effect: Underestimation of high IGF-I levels.



**Figure 1.** Interference of IGFBP in IGF-I measurements. Known concentrations of IGF-I were assayed in the presence of 0.5 ng (left) or 5 ng (right) hIGFBP-3 by a conventional (□) and by the IGFBP-blocked RIA (\*).

Therefore, various techniques were applied to physically separate IGF-I from its binding proteins before measurement, including (a) size exclusion chromatography under acidic conditions, (b) solid-phase extraction and (c) acid-ethanol extraction (2,12,13). These techniques, however, are either inconvenient and time-consuming or give incomplete and not-reproducible recoveries. The most widely-used method is the acid-ethanol extraction (13,14) with a recovery of only 70-80 % of IGFBP-bound IGF-I as a result of co-precipitation. The absolute results of such an extraction are therefore false low (15). The extraction removes the IGFBPs only insufficiently and leads to reduction in sensitivity of the assay due to pre-dilution of the samples by the extraction procedure. Furthermore, the remaining IGFBP may still interfere in the assay. In addition, the acid-ethanol extraction is ineffective in specimens other than serum or plasma (e.g. cell culture media), in which determination of IGF-I is already difficult enough due to the fact that IGFBPs are frequently present at large excess.

To avoid these difficulties, an uncomplicated assay was developed, in which special sample preparation is not required before measurement (except dilution and/or acidification in a specially-composed buffer system).

### Clinical Significance

There are apart from GH, a number of variables that influence serum IGF-I. Decreased levels are found in states of malnutrition/malabsorption, hypothyroidism, liver disease, untreated diabetes mellitus, chronic inflammatory disease (1,6), malignant disease or polytrauma. High levels, on the other hand, are likely to be present in precocious puberty or obesity. Crucially important to the correct interpretation of IGF-I measurements is the relationship between age

and IGF-I levels. It is certainly inadequate to use a common cut-off point to define "normal" levels for all age groups, particularly in children and adolescents (for age-dependant serum levels see table 2 and figures 3-6).

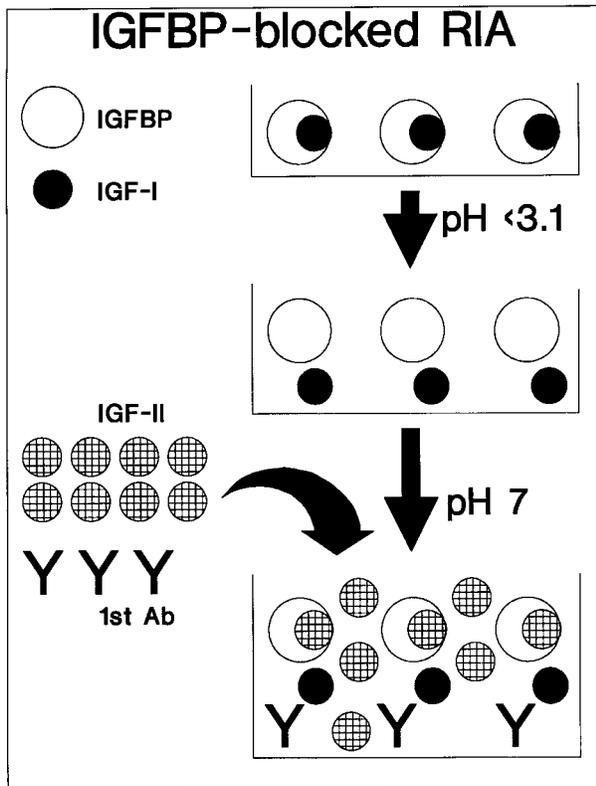
Due to its GH-dependence, determination of serum IGF-I was shown to be a useful tool in diagnosis of growth disorders, especially with regard to GH deficiency (GHD) or acromegaly (6,16-19, 23, 24). The major advantage of IGF-I determination compared to GH determination is its stable circadian concentration, therefore a single measurement is sufficient. Hence IGF-I determination should be the first in a series of laboratory test. Clearly normal levels would then rule out disturbances of the GH-IGF-I-axis. Low levels, i.e. close to or below the age-related 5th percentile would indicate the necessity of further diagnostic efforts. Sub-normal levels of IGF-I would be evidence for reduced GH secretion, if other causes of low serum IGF-I (e.g. malnutrition or impaired liver function) can be ruled out. For differentiation of healthy short children without GH deficiency and children with "classical" GH deficiency, the 0.1st percentile proved to be an appropriate cut-off point, especially after the age of eight. However, IGF-I levels of short children not suffering from GHD may nevertheless lie between the 0.1st and 5th percentile (19). In contrast, acromegaly is characterized by pathologically elevated IGF-I levels which apparently reflect the severity of the disease better than GH-levels (17,18,20).

### **Scientific Use**

IGF-I is present in low concentrations in various body fluids and in conditioned cell culture media of many cell lines. However, the determination of IGF-I in these specimens is particularly difficult due to the presence of IGF-BPs usually in excessive amounts. This explains why conventional assays, in which IGF-BPs are not removed, result in incorrect IGF-I values, which reflect more the present amount of IGF-BP rather than the exact concentration of IGF-I (Figure 1) (15,21). The low IGF-I concentrations require often additional efforts after the extraction procedure to concentrate the extract for obtaining a satisfactory sensitivity. The IGF-BP-blocked IGF-I RIA avoids these problems and allows the simple, correct and sensitive IGF-I determination in numerous samples at the same time.

## ASSAY PRINCIPLE

In order to dissociate IGF-I from the IGFBPs, the samples must be diluted in an acidic buffer (Figure 2). The diluted samples are then pipetted into the assay tubes. The IGF-I antiserum containing an excess of IGF-II is dissolved in a buffer, which is able to neutralize the acidic samples. After the IGF-I antibody solution has neutralized the samples, the excess IGF-II occupies the IGF-binding sites of the binding proteins, thus allowing the measurement of free IGF-I. With this method, the IGFBPs are not removed, but their function and therefore their



interference in the assay is neutralized. Due to the extremely low cross-reactivity of the IGF-I antibody with IGF-II, excess IGF-II does not disturb the interaction of the first antibody with IGF-I or IGF-I tracer. The assay is then continued like a conventional RIA using a second antibody for the separation of bound and free tracer.

The colour of the solutions makes possible for every tube a control of the respective performance step. This enables you to check your pipette plan, if necessary. Dilution and acidification buffer (and, by that, the reconstituted standards and diluted samples too) are coloured in **green** by addition of a pH indicator dye. After addition of the uncoloured IGF-I antibody solution, the now neutralized solutions turn **blue**. Finally, addition of the red coloured tracer solution turns the entire incubation colour **violet**.

Figure 2.: Principle of the IGFBP-blocked IGF-I RIA

## INTENDED USE

This radioimmunoassay kit is suitable for the scientific measurement of IGF-I in human serum or plasma, other human body fluids (e.g. follicular fluid, seminal plasma, urine) or conditioned media of human cell lines. Due to the high cross-reactivity of the first antibody with IGF-I from other mammalian species, it can also be used as a heterologous assay for determination of IGF-I in primates, cattle, pig, sheep, rat and mouse.

## **PRECAUTIONS**

### **General**

All reagents are for Research use only!

In conducting the assay, follow strictly the test protocol. The acquisition, possession and use of the kit is subject to the regulations of the national nuclear regulatory authorities.

Reagents with different lot numbers should not be mixed.

Reagents contain Kathon CG® as preservative. Kathon CG® is toxic when swallowed.

### **The handling of radioactive and potentially infectious material must comply with the following guidelines:**

The material should be stored and used in a special designated area.

Do not eat, drink or smoke in these areas.

Never pipette the materials with the mouth.

Avoid direct contact with these materials by wearing laboratory coats and disposable gloves.

Spilled material must be wiped off immediately. Clean contaminated areas and equipment with a suitable detergent.

Unused radioactive material and radioactive waste should be disposed according to the recommendations of the national regulatory authorities.

### **Radioactivity**

Before ordering or using radioactive materials, it is necessary to take the appropriate actions to ensure compliance with national regulations governing their use. Local rules in each establishment, which define actions and behaviour in the radioactivity working areas, should also be adhered to. The advice given here does not replace any local rules, instructions or training in the establishment, or advice from the radiation protection advisers. It is important to follow the code of good laboratory practice in addition to the specific precautions relating to the radionuclide I-125 used.

Iodine-125 has a radioactive half-life  $T_{1/2}$  of 60 days and emits 35,5 keV gamma radiation, 27 – 32 keV x-rays and no beta radiation. Shielding is effective done by lead, first half value layer is 0.02 mm lead, reduction to 10 % is made by 0.2 mm.

To reduce the radiation dose time spent handling radioactivity should be minimized (plan ahead), and distance from source of radiation should be maximized (doubling the distance from the source quarters the radiation dose).

Formation of aerosols, e.g. by improper opening and mixing of vials or pipetting of solutions which may cause minute droplets of radioactivity become airborne, is a hazard and should be avoided.

Solutions containing iodine should not be made acidic, because this might lead to the formation of volatile elemental iodine.

As some iodo-compounds can penetrate rubber gloves, it is advisable to wear two pairs, or polyethylene gloves over rubber.

For cleaning of contaminated areas or equipment, the Iodine-125 should be rendered chemically stable by using alkaline sodium thiosulphate solution together with paper or cellulose tissue.

## **METHODOLOGY**

### **Assay Characteristics and Validation**

The radioimmunoassay for IGF-I uses a specific, high-affinity polyclonal antibody. Its cross-reactivity with IGF-II is less than 0.05 %. The sensitivity of the assay is 0.02 ng/ml. The tracer is prepared through radioiodination of recombinant hIGF-I. The standards are derived from recombinant hIGF-I devoid of methIGF-I or IGF-I variants with mismatched disulfide bonds, i.e. this recombinant IGF-I is identical to the major authentic IGF-I form in blood. Half-maximal displacement occurs at 1.2 ng/ml. The inter-assay variation coefficient at 50 % B/B0 is 7.4 .

The high sensitivity of the assay allows the measurement of IGF-I in small sample volumes which is limited by pipetting accuracy rather than the amount of IGF-I. Serum or Heparin/EDTA plasma samples must be considerably diluted before measurement. No extraction step is required as in conventional IGF-I assays.

The calibration of the IGF-I radioimmunoassays with regard to the WHO International Reference Standard preparation of IGF-I, NIBSC Code 87/518 (25, 26), yields a conversion factor of: **1.66**, i.e., Mediagnost results must be multiplied by 1.66 to express IGF-I measurements according to the present international standard preparation (it's effective content by respective unitage definition). Please note the controversial discussion regarding its quality, content and reliability (27).

### **Clinical Validation**

Clinical validation was achieved by determining the IGF-I levels in a large number of normal children and adults, normal short statured children without GH deficiency, girls with Ullrich-Turner syndrome, children with Silver-Russell syndrome, patients with GH deficiency, children with familial tall stature, Sotos syndrome, patients with acromegaly, and children with precocious puberty.

### **Validation for Research Purposes**

The presence of IGF-I was demonstrated in a large number of samples, e.g. follicular fluid, seminal plasma, urine, and in conditioned media from various cell-lines of mammalian origin.

### **Sample Preparation and Storage**

The stability of IGFBP-bound IGF-I makes sample preparation simple. Serum and Heparin/EDTA plasma levels are comparable. Blood samples may be taken at any time of the day. Whole blood should be processed within a few hours and stored frozen at -20°C until measurement. IGF-I levels are usually not affected by improper handling or storage. They remain stable over several days in normal and in various clinical situations even under conditions of high temperature (37°C). Avoid repeated freezing and thawing cycles, although IGF-levels in normal sera remained unchanged after 10 cycles. Frozen samples are stable over many years. Samples may also be freeze-dried without suffering any loss of activity.

Sample requirements: 10 µl serum or plasma (minimum 5µl).

## MATERIALS

### Materials provided

The reagents listed below are sufficient for 100 tubes including the standard curve.

- AB** Acidification Buffer  
(1 bottle, 12.5 ml, ready for use, coloured)
- DB** Dilution Buffer  
(2 bottles, 125 ml, ready for use, coloured)
- A** Assay Buffer  
(2 bottles, 30 ml, ready for use)
- B** 1st Antibody (anti-hIGF-I) containing rabbit IgG and rec hIGF-II  
(2 bottles, 11 ml, lyophilized)
- C** Tracer:  $^{125}\text{I}$ -IGF-I;  $< 1.30 \mu\text{Ci}$  or  $< 50 \text{ kBq}$   
(2 bottles, 11 ml, lyophilized, red coloured)
- D** Rabbit immunoglobulin for non-specific binding (NSB)  
(1 vial, 500  $\mu\text{l}$ , lyophilized)
- E - L** Standards: Concentrations given on vial-labels in ng/ml  
(8 vials, 500  $\mu\text{l}$  each, lyophilized)
- M+N** Controls (human serum): Conc. given on vial-labels in ng/ml  
(2 vials, 100  $\mu\text{l}$  each, lyophilized)
- O** 2nd Antibody (anti-rabbit immunoglobulin)  
(2 vials, 1 ml, lyophilized)
- P** Precipitation Reagent  
(2 bottles, 55 ml, ready for use after adding O)

### Reagent Storage

Store the kit at **2-8°C** after receipt until its expiry date. The lyophilized reagents should be stored at **-20 °C** after reconstitution. Avoid repeated thawing and freezing. The shelf-life of the reagents after opening is not affected, if used appropriately.

### Required Materials Not Provided

- 1) Ice-cold deionized water
- 2) Pipettes: 10 ml, 1 ml, 500  $\mu\text{l}$ , 250  $\mu\text{l}$ , 100  $\mu\text{l}$ , 10  $\mu\text{l}$ ;  
100  $\mu\text{l}$ , 250  $\mu\text{l}$  and 1 ml repeating pipettes are recommended.
- 3) Disposable polystyrene or polypropylene tubes. Conical tubes are highly recommended because of the small immunoprecipitates.  
The use of round-bottom tubes may cause formation of insufficiently compact pellets.
- 4) Vortex mixer
- 5) Centrifuge appropriate for precipitation of immunocomplexes (ideally with cooling).
- 6) Device for aspiration of liquid supernatant (e.g. connected to a water pump).
- 7) Gamma counter

## Reagent Preparation

**B + C** Reconstitute with **11 ml** reagent **A** (Assay Buffer).

**D** Reconstitute with **500 µl** reagent **A** (Assay Buffer).

**E - L** Reconstitute with **500 µl** reagent **DB** (Dilution Buffer).

**M+N** Reconstitute with **100 µl distilled water**.

Further dilution according to sample dilution with **DB** (e.g. 1:101)

**O** Reconstitute with **1 ml** reagent **A**. Transfer dissolved material to reagent P immediately before use. For 100 tubes add 1 vial reagent O (reconstituted in 1 ml A) to 1 bottle of reagent P (55 ml) or any volumes in the same ratio (1:56) for fewer tubes. The assay is unaffected by the possible occurrence of turbidity in the final reagent.

Ensure that lyophilized materials are completely dissolved on reconstitution. It is recommended to keep reconstituted reagents at **room temperature** for **half an hour** and then to mix them vigorously with a Vortex mixer. This is important in particular for the **controls M and N** !

## Sample Preparation

Serum or plasma samples should be diluted depending on the expected values 1:30 -1:400 with **Dilution Buffer DB**. Usually, a dilution of 1:100 - 1:150 is appropriate.

Example: Add 10 µl serum to 1 ml **Dilution Buffer DB** (dilution 1:101).

If very low levels are expected (e.g. in extreme GH deficiency or in GH receptor deficiency), serum or plasma samples may be diluted 1:20 or less with **Dilution Buffer DB**. Sufficient acidification can be achieved by adding **Acidification Buffer AB** (1/10th of the diluted sample volume).

Example: Dilute 10 µl serum with 200 µl **Dilution Buffer DB** (1:21). Add 20 µl **Acidification Buffer AB** (total dilution 1:23).

In body fluids other than serum or plasma (e.g. cerebrospinal fluid, ocular vitreous fluid, urine, or conditioned cell culture media), IGF-I concentrations may be extremely low. These samples can be measured directly without dilution after adding 1/10th of their volume **Acidification Buffer AB**

Example: Add 20 µl **Acidification Buffer AB** to 200 µl conditioned cell culture medium (dilution 10:11, dilution factor: 1.1).

The dilution of the controls (**M and N**) with **Dilution Buffer DB** should be according to the common dilution of serum or plasma samples, e.g. about 1:101.

## ASSAY PROCEDURE

Flow Chart of Assay Protocol:

#	Tube	DB	E-L M,N Patients	D	B	C	P
1,2	Total	---	---	---	---	100	---
3,4	NSB	100	---	100	---	100	500
5,6	B <sub>0</sub>	---	100 E	---	100	100	500
7-20	Standards	---	100 F-L	---	100	100	500
21,22	High Control	---	100 M	---	100	100	500
23,24	Low Control	---	100 N	---	100	100	500
25,26	Sample 1	---	100	---	100	100	500
27,28	Sample 2	---	100	---	100	100	500
Etc.							
<b>Colour :</b>			<b>Green</b>		After addition: <b>Blue</b>	After addition: <b>Violet</b>	

All volumes are given as  $\mu\text{l}$ .

Samples (standards and patient samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the test-protocol are recommended.

- 1) Labelling of the assay tubes (duplicates) should be done in the following order: 1 and 2 **total counts**, 3 and 4 **NSB**, 5 and 6 **zero standard (B<sub>0</sub>)**, 7 to 20 **standards**, 21 to 24 controls, 25 to 100 **samples**.
- 2) Add 100  $\mu\text{l}$  of Dilution Buffer **DB** to tubes 3 and 4.
- 3) Add 100  $\mu\text{l}$  of reagents **E - L (standards)** to tubes 5 to 20, (**zero standard (E)** to tubes 5 and 6, **standard F** (0.156 ng/ml) to tubes 7 and 8, etc).
- 4) Add **100  $\mu\text{l}$**  of diluted reagent **M (high control)** to tubes 21 and 22 and **100  $\mu\text{l}$**  of diluted reagent **N (low control)** to tubes 23 and 24.
- 5) Add **100  $\mu\text{l}$**  of diluted (or only acidified) **samples** to tubes 25 and 26, etc.  
-All solutions appear **green!**-
- 6) Add **100  $\mu\text{l}$**  reagent **D (NSB)** to tubes 3 and 4.
- 7) Add **100  $\mu\text{l}$**  reagent **B (1st Antibody)** beginning with tube 5.  
-All solutions turn **blue!**-
- 8) Add **100  $\mu\text{l}$**  reagent **C** (tracer) to all tubes.  
-All solutions turn **violet!**-
- 9) Remove tubes 1 and 2 (**total counts**) or mark or seal with a stopper.
- 10) Mix tubes with a vortex mixer.

- 11) Incubate tubes at **2 - 8 °C** for **2 days**. Incubation for a longer period (e.g. **over the weekend**) has no negative effect on the results. Incubation for a shorter period (e.g. **overnight**) leads to a weaker bondage resulting in a slight loss of sensitivity, irrelevant for most routine measurements.
- 12) Add **500 µl** reagent **P** (after addition of reagent **O** !), beginning with tube 3. The reagent should be cold (**2 - 8 °C**).
- 13) Mix tubes with a vortex mixer.
- 14) Incubate tubes at **2 - 8 °C** for **1 hour**.
- 15) Add **1 ml ice-cold distilled water**.
- 16) Centrifuge all tubes except tubes 1 and 2 at least at **3000 x g** for **30 min** at a temperature of **2 - 8 °C**.
- 17) Aspirate the supernatant (except tubes 1 and 2 !). The remaining supernatant should not be higher than 5 mm above the precipitate. Take care that the precipitate remains intact. Depending on local conditions and procedures, the supernatant may also be decanted instead of aspirated.
- 18) Count the activity of **all** tubes (including tubes 1 and 2) for **1 to 3 min**.

#### **Extended washing procedure for increased precision**

The second incubation step (step 14) is directly followed by step 16 (centrifugation) and step 17 (aspiration). Proceed then with step 15 and add 1 ml of ice-cold water. This should not be done too vigorously in order to keep the precipitate intact. **Do not mix again!** Centrifuge the tubes at 2-8°C at 3000 x g for 5 min., aspirate the supernatant, and count the radioactivity of all tubes in the gamma-counter (step 18).

This extended procedure results in a somewhat higher precision and reduces the non-specific binding NSB. This is also bound up with a higher work expenditure. The higher precision may be irrelevant for most measurements and should therefore be used only in special cases.

## **EVALUATION OF RESULTS**

### **Establishing of the Standard Curve**

The standards provided contain the following concentrations of IGF-I :

Standard	E	F	G	H	I	J	K	L
ng/ml	0.0	0.156	0.313	0.625	1.25	2.5	5.0	10

1. Calculate the average counts of each pair of tubes.
2. Subtract the average counts of NSB tubes (3 and 4) from the mean counts of the standards, controls and patient samples. This gives the corrected values for B.
3. The corrected value from the zero standard E(tubes 5 and 6) is  $B_0$ .
4. Calculate the percent bound (% B/ $B_0$ ) by dividing the corrected B-values by  $B_0$ :  $B/B_0 \times 100\%$ .
5. Plot % B/ $B_0$  versus the standard concentrations on either semi-logarithmic or logit-log paper. For convenience, it is recommended to use computer assisted data reduction programs.

6. For quality control calculate NSB in %: average counts of tubes 3 and 4 divided by the average counts of tubes 1 and 2 (Total Count, TC) times 100%. It should be < 3% (%NSB/TC < 3).

Calculate the percent bound of the zero standard E: average counts of tubes 5 and 6 minus average counts of NSB divided by TC times 100%. It should be > 30% (%B<sub>0</sub>/TC > 30).

### Evaluation of sample concentrations:

Read the concentration value (abscissa) corresponding to the % B/B<sub>0</sub> of the sample as in the example given below:

average counts of NSB: 156 cpm  
average counts of zero standard (B<sub>0</sub>): 9005 cpm  
average counts of sample: 3812 cpm

$$\begin{aligned}\%B/B_0 &= (\text{sample-counts} - \text{NSB}) / (B_0 - \text{NSB}) \times 100\% \\ &= (3812 - 156) / (9005 - 156) \times 100\% \\ &= 0.413 \times 100\% \\ &= 41.3\%\end{aligned}$$

For a 41.3 % value on the y-axis (ordinate) a value of 1.52 ng/ml on the x-axis (abscissa) was obtained. Multiply the concentration value determined graphically or by the aid of a computer program with the dilution factor.

Example: 1.52 x 101 = 154 ng/ml.

If it is preferred to express the results as nmol/l, the values given as ng/ml should be divided by 7.649 to obtain nmol/l.

Example: 154 ng/ml : 7.649 = 20 nmol/l

### Concentration of control samples

The IGF-I concentration of Controls M and N should be within the following range (mean value ± 2 SD):

High Control M: **203 ± 31 ng/ml**

Low Control N: **61±12 ng/ml**

### EXPECTED VALUES

IGF-I levels are highly age-dependent in children, less so in adults until the age of about 60. The normal ranges in various age groups, which are log-normally distributed, are given in Table 2 by percentiles. Between 8 and 19 years of age, values are given for boys and girls separately, because the pubertal peak usually occurs approximately 2 years earlier in girls. A graphic presentation is shown in Figures 3, 4 and 5. A major problem for the interpretation of IGF-I values arises from the fact, that short stature is often due to developmental delay rather than any metabolic or endocrine disorder (constitutional delay of growth and adolescence). The sharp rise in IGF-I levels during puberty may therefore cause some uncertainty as to whether or not it would be appropriate to relate measured values to chronological age. It is

recommended to take the pubertal stage into account (Table 1 and Figure 6) to get a more complete picture of this situation.

**Table 1:** Normal range of serum IGF-I levels at different pubertal stages according to Tanner. Because no significant difference between boys and girls is observed, both sexes are combined. Only children and adolescents between 7 and 17 years of age are included.

Pubertal Stage	Percentile			
	0.1th	5th	50th	95th
1	61	105	186	330
2	85	156	298	568
3	113	196	352	631
4	171	268	431	693
5	165	263	431	706

## LIMITATIONS

IGF-I levels depend to a great degree on GH secretion. Diminished IGF-I values, however, do not prove GH deficiency, because a number of other factors can influence the plasma concentration of IGF-I and must therefore be taken into account in order to make a correct interpretation. IGF-I levels decrease during fasting (more than 1 day), as a result of malnutrition, malabsorption, cachexia, impaired hepatic function, or in hypothyroidism and untreated diabetes mellitus. They may also be low in chronic inflammatory disease and malignancies. IGF-I levels are high in states of accelerated sexual development. In clinical situations with hyperprolactinemia or in patients with craniopharyngioma, normal levels may be observed despite GH deficiency. In late pregnancy, IGF-I levels are moderately elevated.

**Tab.2:** Serum levels of IGF-I in healthy subjects at various ages. Individuals between 8 and 19 years of age were classified according to gender, as the pubertal peak occurs almost 2 years earlier in girls than in boys.

Age	Percentile													
	0.1	1	5	10	20	30	40	50	60	70	80	90	95	99
0-2 y.	<b>13</b>	20	<b>28</b>	34	<b>43</b>	50	<b>58</b>	66	<b>75</b>	87	<b>102</b>	128	<b>156</b>	220
2-4 y.	<b>20</b>	29	<b>40</b>	48	<b>59</b>	68	<b>77</b>	87	<b>98</b>	111	<b>129</b>	159	<b>189</b>	260
4-6 y.	<b>26</b>	36	<b>50</b>	59	<b>73</b>	85	<b>96</b>	108	<b>122</b>	138	<b>160</b>	196	<b>233</b>	320
6-7 y.	<b>34</b>	46	<b>62</b>	72	<b>87</b>	99	<b>111</b>	124	<b>138</b>	155	<b>176</b>	212	<b>248</b>	332
7-8 y.	<b>45</b>	60	<b>78</b>	90	<b>107</b>	121	<b>134</b>	148	<b>163</b>	181	<b>205</b>	243	<b>281</b>	364
8-9 y. boys	<b>54</b>	71	<b>90</b>	102	<b>119</b>	133	<b>146</b>	160	<b>175</b>	192	<b>214</b>	250	<b>284</b>	362
8-9 y. girls	<b>55</b>	75	<b>99</b>	115	<b>137</b>	156	<b>174</b>	193	<b>214</b>	239	<b>271</b>	323	<b>369</b>	495
9-10 y. boys	<b>63</b>	82	<b>102</b>	115	<b>133</b>	148	<b>162</b>	176	<b>191</b>	209	<b>232</b>	269	<b>304</b>	379
9-10 y. girls	<b>68</b>	89	<b>114</b>	130	<b>152</b>	170	<b>187</b>	205	<b>224</b>	247	<b>276</b>	324	<b>376</b>	470
10-11 y. boys	<b>77</b>	96	<b>117</b>	130	<b>148</b>	162	<b>176</b>	189	<b>203</b>	220	<b>241</b>	274	<b>305</b>	370
10-11 y. girls	<b>81</b>	106	<b>134</b>	153	<b>178</b>	199	<b>219</b>	239	<b>261</b>	287	<b>321</b>	374	<b>426</b>	539
11-12 y. boys	<b>85</b>	106	<b>129</b>	144	<b>163</b>	179	<b>194</b>	209	<b>225</b>	244	<b>267</b>	304	<b>339</b>	413
11-12 y. girls	<b>91</b>	123	<b>160</b>	185	<b>220</b>	248	<b>276</b>	305	<b>337</b>	374	<b>424</b>	503	<b>581</b>	758
12-13 y. boys	<b>88</b>	112	<b>141</b>	159	<b>184</b>	204	<b>223</b>	243	<b>264</b>	289	<b>321</b>	371	<b>419</b>	525
12-13 y. girls	<b>116</b>	155	<b>201</b>	231	<b>274</b>	309	<b>342</b>	377	<b>415</b>	460	<b>519</b>	614	<b>707</b>	914
13-14 y. boys	<b>111</b>	143	<b>179</b>	203	<b>235</b>	261	<b>286</b>	311	<b>339</b>	371	<b>412</b>	477	<b>540</b>	677
13-14 y. girls	<b>163</b>	207	<b>256</b>	287	<b>329</b>	364	<b>395</b>	428	<b>463</b>	504	<b>556</b>	637	<b>716</b>	884
14-15 y. boys	<b>140</b>	182	<b>229</b>	260	<b>303</b>	337	<b>370</b>	404	<b>441</b>	484	<b>539</b>	625	<b>691</b>	896
14-15 y. girls	<b>193</b>	236	<b>284</b>	314	<b>353</b>	385	<b>414</b>	443	<b>474</b>	510	<b>556</b>	628	<b>713</b>	832
15-16 y. boys	<b>176</b>	221	<b>269</b>	299	<b>340</b>	372	<b>402</b>	433	<b>466</b>	504	<b>552</b>	626	<b>697</b>	849
15-16 y. girls	<b>187</b>	231	<b>279</b>	309	<b>350</b>	382	<b>412</b>	442	<b>474</b>	512	<b>559</b>	632	<b>700</b>	845
16-17 y. boys	<b>178</b>	221	<b>267</b>	296	<b>335</b>	366	<b>395</b>	424	<b>455</b>	491	<b>537</b>	607	<b>673</b>	814
16-17 y. girls	<b>183</b>	225	<b>270</b>	298	<b>336</b>	366	<b>394</b>	422	<b>452</b>	486	<b>530</b>	597	<b>660</b>	792
17-18 y. boys	<b>173</b>	207	<b>243</b>	265	<b>294</b>	317	<b>337</b>	358	<b>380</b>	405	<b>436</b>	484	<b>527</b>	618
17-18 y. girls	<b>176</b>	210	<b>246</b>	268	<b>297</b>	320	<b>341</b>	362	<b>384</b>	409	<b>441</b>	488	<b>533</b>	624
18-19 y. boys	<b>167</b>	201	<b>235</b>	256	<b>285</b>	307	<b>327</b>	347	<b>368</b>	393	<b>423</b>	469	<b>512</b>	600
18-19 y. girls	<b>167</b>	146	<b>233</b>	254	<b>281</b>	302	<b>322</b>	341	<b>362</b>	385	<b>414</b>	458	<b>499</b>	797
19-20 y.	<b>158</b>	189	<b>220</b>	240	<b>265</b>	285	<b>304</b>	322	<b>341</b>	363	<b>391</b>	433	<b>471</b>	550
20-30 y.	<b>72</b>	92	<b>115</b>	130	<b>150</b>	167	<b>182</b>	198	<b>215</b>	235	<b>261</b>	302	<b>340</b>	425
30-40 y.	<b>68</b>	87	<b>109</b>	123	<b>142</b>	158	<b>173</b>	188	<b>204</b>	223	<b>248</b>	287	<b>324</b>	404
40-50 y.	<b>64</b>	82	<b>103</b>	116	<b>135</b>	150	<b>164</b>	178	<b>194</b>	212	<b>235</b>	272	<b>310</b>	385
50-60 y.	<b>60</b>	77	<b>97</b>	110	<b>127</b>	142	<b>155</b>	169	<b>184</b>	201	<b>224</b>	260	<b>292</b>	369
60-70 y.	<b>55</b>	72	<b>91</b>	103	<b>120</b>	134	<b>147</b>	161	<b>176</b>	193	<b>215</b>	251	<b>282</b>	362
70-80 y.	<b>25</b>	35	<b>47</b>	55	<b>67</b>	78	<b>88</b>	98	<b>110</b>	124	<b>142</b>	173	<b>207</b>	276
> 80 y.	<b>21</b>	50	<b>40</b>	47	<b>58</b>	67	<b>76</b>	85	<b>95</b>	108	125	153	<b>184</b>	245

Serum concentrations are given in ng/ml.

Determined with IGFBP-blocked IGF-I RIA without extraction step (Blum and Breier 1994).

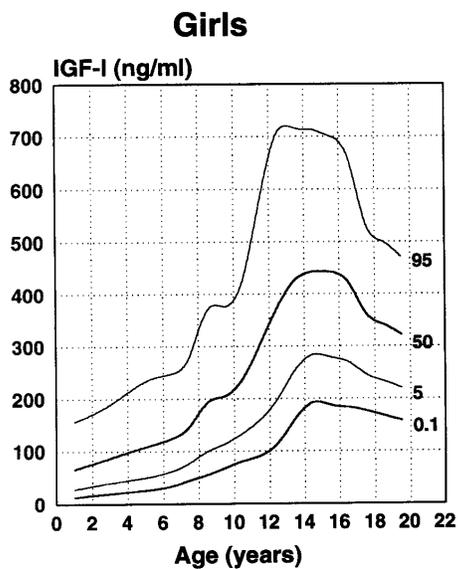


Fig. 3: Age-dependent normal range of serum IGF-I levels in girls

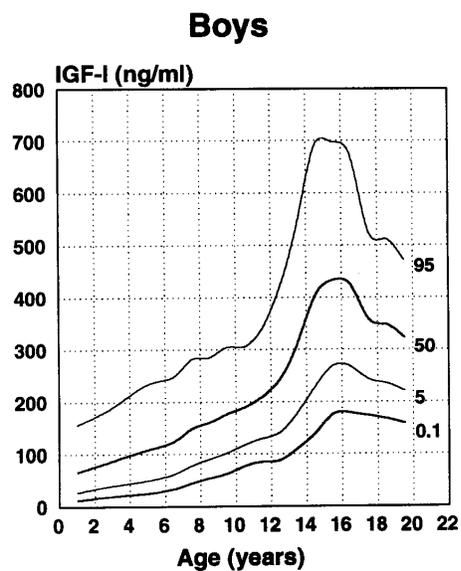


Fig. 4: Age-dependent normal range of serum IGF-I levels in boys

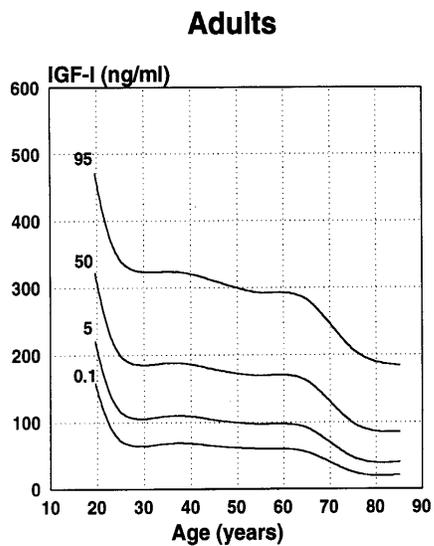


Fig. 5: Age-dependant normal range of serum IGF-I levels in adults

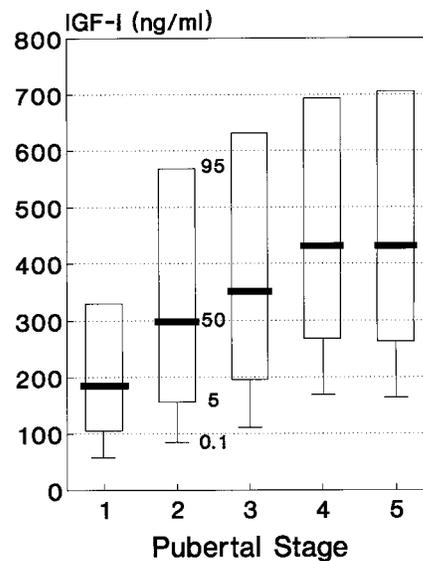


Fig 6.: Serum IGF-I levels in normal children and adolescents (7 to 17 years) according to pupertal stages. Both sexes were included.

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