IGF-I RIA

Radioimmunoassay for Quantitative Determination of

Insulin-like Growth Factor-I

(IGFBP blocked)

Product Code: IGF-R20

(for 100 tubes)

For In Vitro Diagnostic Use

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INTENDED USE

A radioimmunoassay for the quantitative *in vitro diagnostic* measurement of levels of IGF-I in serum or plasma. Human IGF-I measurements are used in the diagnosis and treatment of growth disorders involving the anterior lobe of the pituitary gland.

SUMMARY AND EXPLANATION

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with high IGF-I concentration, unmarked IGF-I from the sample will be predominantly complexed by IGFBPs and therefore withdrawn from measurement.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

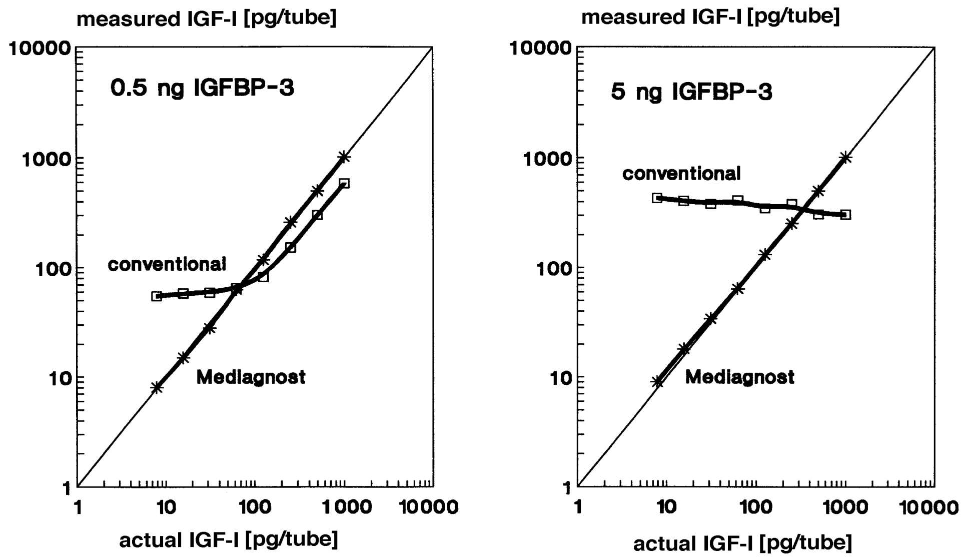

Figure 1. Interference of IGFBP in IGF-I measurements. Known concentrations of IGF-I were assayed in the presence of 0.5 ng (left) or 5 ng (right) hIGFBP-3 by a conventional (•)and by the IGFBP-blocked RIA (*).

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 or 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.

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.

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. Subnormal 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 lay 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).

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.

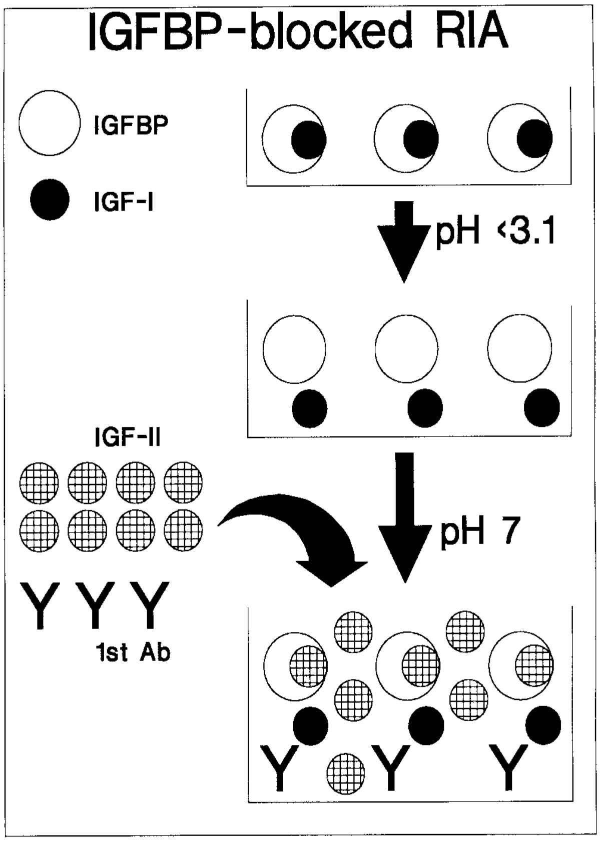

Figure 2: Principle of the IGFBP- blocked IGF-I RIA

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 (including 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**.

WARNINGS AND PRECAUTIONS

1. For *In Vitro Diagnostic Use*.
2. For professional use only.
3. The acquisition, possession and use of the kit are subject to the regulations of the national nuclear regulatory authorities.
4. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
5. Before use, all kit components should be brought to room temperature at 68-77°F (20 - 25°C). Precipitates in buffers should be dissolved before use by thorough mixing and warming. Temperature WILL affect the counts of the assay. However, values for the patient samples will not be affected.
6. Do not mix reagents of different lots. Do not use expired reagents.
7. Reagents contain Sodium-Azide (0.02%) as preservative. Sodium-Azide is very toxic, therefore, R-Phrases: 28, 32, 50/53 and S-Phrases 28, 45, 60, and 61 must be considered.
8. **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 behavior 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 Iodine-125 used.

Iodine-125 has a radioactive half-life T1/2 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 of gloves, 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.

<u>General First Aid Procedures</u>:

Skin contact: Wash affected area thoroughly with water at least 15 minutes. Discard contaminated cloths and shoes. See a physician.

Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing spread the eyelids. See a physician.

Ingestion: If swallowed, wash out mouth thoroughly with water, provided that the person is conscious. Immediately see a physician.

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.

REAGENTS 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 - (1 bottle, 125 mL, ready for use, coloured)

A Assay Buffer - (1 bottle, 30 mL, ready for use)

B 1st Antibody (anti-hIGF-I) containing rabbit IgG and rec hIGF-II - (1 bottle, 11 mL, lyophilized)

C Tracer: ^{125}I-IGF-I; < 1.5 µCi or < 55 kBq - (1 bottle, 11 mL, lyophilized, red coloured)

D Rabbit immunoglobulin for non-specific binding (NSB) - (1 vial, 500 µL, lyophilized)

E - L Standards: Concentrations given on vial-labels in ng/mL - (8 vials, 500 µL each, lyophilized)

M+N Controls (human serum): Conc. given on vial-labels in ng/mL - (2 vials, 100 µL each, lyophilized)

O 2nd Antibody (anti-rabbit immunoglobulin) - (1vial, 1 mL, lyophilized)

P Precipitation Reagent - (1 bottle, 55 mL, ready for use after adding O)

MATERIALS REQUIRED BUT NOT PROVIDED

1) Ice-cold deionized water

2) Pipettes: 10 mL, 1 mL, 500 µL, 250 µL, 100 µL, 10 µL;
100 µL, 250 µL 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 or more tubes. The assay is unaffected by the possible occurrence of turbidity in the final reagent.

Note: 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**!

STORAGE CONDITIONS

Store the kit at **35.6- 46.4°F** (**2-8°C)** after receipt until its expiry date. The lyophilized reagents should be stored at –4°F (**–20 °**C) after reconstitution. Avoid repeated thawing and freezing. The shelf-life of the reagents after opening is not affected, if used appropriately.

SPECIMEN COLLECTION, PREPARATION, AND STORAGE

Serum and Heparin/EDTA plasma levels are comparable. Citrated Plasma levels are reduced, because the anticoagulant amount dilutes the small sample slightly. Blood samples may be taken at any time of the day. Whole blood should be processed within a few hours and stored frozen at -4°F (-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 (98.6°F/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 5 µL).

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 vitrous 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:

Nr. of tubes	Contents of tube	DB E-L M,N Samples	D	B	C	P
1, 2	Total Counts	---	---	---	100	---
3, 4	NSB	100 **DB**	100	---	100	500
5, 6	B_0	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.						
Colour after addition:		**Green**		**Blue**	**Violet**	

Note: All volumes are given as µL.

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_0**), 7 to 20 **standards**, 21 to 24 controls, 25 to 100 **samples**.
2) Add **100 µL** of Dilution Buffer **DB** to tubes 3 and 4.
3) Add **100 µL** 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 µL** of diluted reagent **M** (**high control**) to tubes 21 and 22 and **100 µL** of diluted reagent **N** (**low control**) to tubes 23 and 24.
5) Add **100 µL** of diluted (or only acidified) **samples** to tubes 25 and 26, etc. - All solutions appear **green**!

6) Add **100 µL** reagent **D (NSB)** to tubes 3 and 4.
7) Add **100 µL** reagent **B (1st Antibody)** beginning with tube 5. - All solutions turn **blue**!
8) Add **100 µL** 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 **35.6-46.4°F (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** (<u>after</u> addition of reagent **O**), beginning with tube 3. The reagent should be cold **35.6-46.4°F** (2 - 8 °C).
13) Mix tubes with a vortex mixer.
14) Incubate tubes at **35.6- 46.4°F** (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 **35.6- 46.4°F (**2 - 8 °C**)**.
17) Aspirate the supernatant (except tubes 1 and 2 !). The remaining supernatant should not be higher than 2 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**.

QUALITY CONTROL

The handling of radioactive and potentially infectious material must comply with Good laboratory practice (GLP). GLP requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state or local standards/laws. All standards and kit controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

CALCULATION OF RESULTS

Assay Characteristics and Validation

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 1.8 ng/mL.

This radioimmunoassays is calibrated against the WHO International Reference Standard preparation of IGF-I, **WHO NIBSC 02/254** (25-26).

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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 x 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 < 5% (%NSB/TC< 5).

   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_0/TC > 30).

Example of Typical Standard Curve

The following data is for demonstration only and cannot be used in place of data generation at the time of assay.

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
cpm	9721.5	8930.4	8410.5	7395.1	6046.2	4484.3	2999.9	2074.8

Evaluation of sample concentrations:

Read the concentration value (abscissa) corresponding to the % B/B_0 of the sample as in the example given below:

average counts of NSB: 484.5 cpm

average counts of zero standard (B_0): 9721.5 cpm

average counts of sample: 4946.6 cpm

$\%B/B_0$ = (sample-counts - NSB) / (B_0 - NSB) x 100%

= (4946.6 – 484.5) / (9721.5 – 484.5) x 100%

= 0.483 x 100%

= 48.3%

For a 48.3 % value on the y-axis (ordinate) a value of 2.01 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: 2.01 x 101 = 203 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: 203 ng/mL : 7.649 = 26.5 nmol/L

Concentration of control samples

The IGF-I concentrations of Controls **M & N** should be within the ranges given on the vial labels.

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.

	Percentile			
Pubertal Stage	**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

Table 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.

		Percentile													
Age		**0.1**	1	**5**	10	**20**	30	**40**	50	**60**	70	**80**	90	**95**	99
0-2 y.		**13**	20	**28**	34	**43**	50	**58**	66	**75**	87	**102**	128	**156**	220
2-4 y.		**20**	29	**40**	48	**59**	68	**77**	87	**98**	111	**129**	159	**189**	260
4-6 y.		**26**	36	**50**	59	**73**	85	**96**	108	**122**	138	**160**	196	**233**	320
6-7 y.		**34**	46	**62**	72	**87**	99	**111**	124	**138**	155	**176**	212	**248**	332
7-8 y.		**45**	60	**78**	90	**107**	121	**134**	148	**163**	181	**205**	243	**281**	364
8-9 y.	boys	**54**	71	**90**	102	**119**	133	**146**	160	**175**	192	**214**	250	**284**	362
	girls	**55**	75	**99**	115	**137**	156	**174**	193	**214**	239	**271**	324	**376**	496
9-10 y.	boys	**63**	82	**102**	115	**133**	148	**162**	176	**191**	209	**232**	269	**304**	379
	girls	**68**	89	**114**	130	**152**	170	**187**	205	**224**	247	**276**	323	**369**	469
10-11 y.	boys	**77**	96	**117**	130	**148**	162	**176**	189	**203**	220	**241**	274	**305**	370
	girls	**81**	106	**134**	153	**178**	199	**219**	239	**261**	287	**321**	374	**426**	539
11-12 y.	boys	**85**	106	**129**	144	**163**	179	**194**	209	**225**	244	**267**	304	**339**	413
	girls	**91**	123	**160**	185	**220**	248	**276**	305	**337**	374	**424**	503	**581**	758
12-13 y.	boys	**88**	112	**141**	159	**184**	204	**223**	243	**264**	289	**321**	371	**419**	525
	girls	**116**	155	**201**	231	**274**	309	**342**	377	**415**	460	**519**	614	**707**	914
13-14 y.	boys	**111**	143	**179**	203	**235**	261	**286**	311	**339**	371	**412**	477	**540**	677
	girls	**163**	207	**256**	287	**329**	364	**395**	428	**463**	504	**556**	637	**716**	884
14-15 y.	boys	**140**	182	**229**	260	**303**	337	**370**	404	**441**	484	**539**	625	**691**	896
	girls	**193**	236	**284**	314	**353**	385	**414**	443	**474**	510	**556**	628	**713**	832
15-16 y.	boys	**176**	221	**269**	299	**340**	372	**402**	433	**466**	504	**552**	626	**697**	849
	girls	**187**	231	**279**	309	**350**	382	**412**	442	**474**	512	**559**	632	**700**	845
16-17 y.	boys	**178**	221	**267**	296	**335**	366	**395**	424	**455**	491	**537**	607	**673**	814
	girls	**183**	225	**270**	298	**336**	366	**394**	422	**452**	486	**530**	597	**660**	792
17-18 y.	boys	**173**	207	**243**	265	**294**	317	**337**	358	**380**	405	**436**	484	**527**	618
	girls	**176**	210	**246**	268	**297**	320	**341**	362	**384**	409	**441**	488	**533**	624
18-19 y.	boys	**167**	201	**235**	256	**285**	307	**327**	347	**368**	393	**423**	469	**512**	600
	girls	**167**	199	**233**	254	**281**	302	**322**	341	**362**	385	**414**	458	**499**	583
19-20 y.		**158**	189	**220**	240	**265**	285	**304**	322	**341**	363	**391**	433	**471**	550
20-30 y.		**72**	92	**115**	130	**150**	167	**182**	198	**215**	235	**261**	302	**340**	425
30-40 y.		**68**	87	**109**	123	**142**	158	**173**	188	**204**	223	**248**	287	**324**	404
40-50 y.		**64**	82	**103**	116	**135**	150	**164**	178	**194**	212	**235**	272	**310**	385
50-60 y.		**60**	77	**97**	110	**127**	142	**155**	169	**184**	201	**224**	260	**292**	369
60-70 y.		**55**	72	**91**	103	**120**	134	**147**	161	**176**	193	**215**	251	**282**	362
70-80 y.		**25**	35	**47**	55	**67**	78	**88**	98	**110**	124	**142**	173	**207**	276
>80 y.		**21**	30	**40**	47	**58**	67	**76**	85	**95**	108	**125**	153	**184**	245

Serum concentrations are given in ng/mL.

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

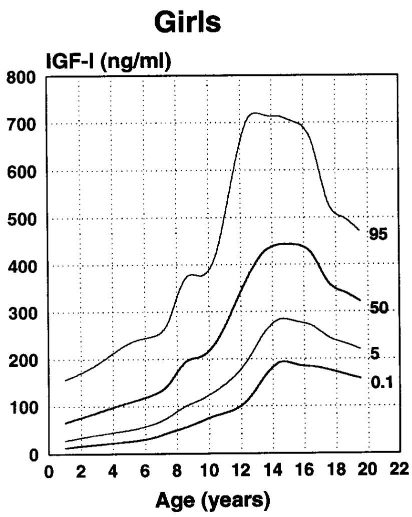

Fig. 3: Age-dependent normalrange of serum IGF-I levels in girls

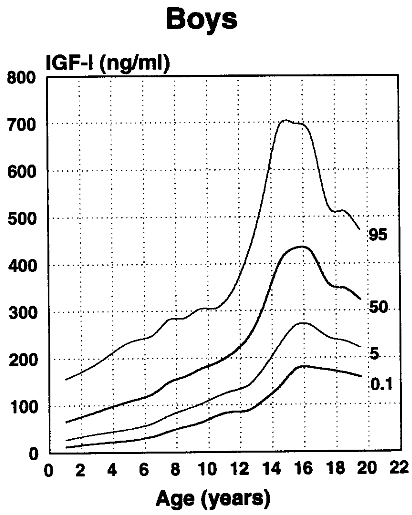

Fig. 4: Age-dependent normal range of serum IGF-I levels in boys

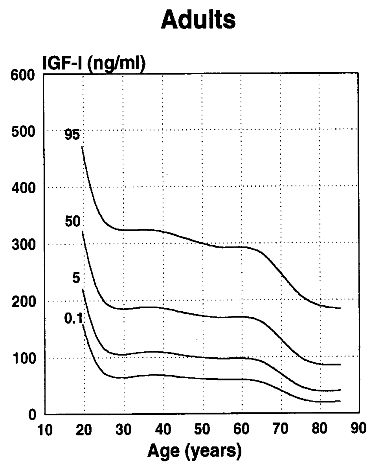

Fig. 5: Age-dependant normal range of serum IGF-I levels in adults

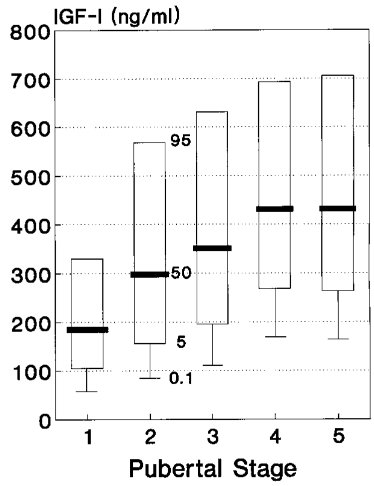

Fig 6.: Serum IGF-I levels in normal children and adolescents (7 to 17 years) according to pubertal stages. Both sexes were included.

LIMITATIONS OF PROCEDURE

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.

PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical sensitivity of the radioimmunoassay for IGF-I R20 yields 0.02 ng/mL 2x SD of zero standards in 15-fold determination.

Specificity

The following materials have been evaluated for cross reactivity. 200 ng/mL solutions of each substance have been analysed in this Radioimmunoassay.

	IGF-II	Insulin	Proinsulin	C-Peptide
Reactivity [%]	0.05	0.005	0.012	0.019

Reproducibility

Intra-Assay-Variation

	Number of determinations	Mean value (ng/mL)	Standard deviation (ng/mL)	CV (%)
Sample 1	6	378	9.84	2.61
Sample 2	6	130	5.37	4.12
Sample 3	6	149	4.65	3.12

Inter-Assay-Variation

The inter-assay variation coefficient at 50 % B/B_0 is 5.5%

		Mean value (ng/mL)	Standard deviation (ng/mL)	CV (%)
Sample 1	17	509	17.3	3.4
Sample 2	16	136	5.7	4.2
Sample 3	17	63	2.6	4.1

Linearity

Dilution:	Sample 1 (calculated, ng/mL)	Dilution:	Sample 2 (calculated, ng/mL)
1:50	501.5	1:15	141.7
1:100	524.1	1:30	161.1
1:200	541.0	1:60	169.8
1:400	548	1:120	170.8
1:800	545.6	1:240	174.5
1:1600	547.2	1:480	174.7
AV / 1SD	535 / 18.5	AV / 1SD	165,43/ 12.63

AV = Average Value , SD = Standard Deviation

Recovery

The **recovery** of the recombinant IGF-I yielded in a buffer matrix 100%. In different human-sera the recovery was on average 103 % of the hypothetical expected amount.

100 ng/mL IGF-I added	Buffer	Serum 1	Serum 2	Serum 3
Recovery [%]	100	116.3	101	95.7

COMPARISON STUDIES

Studies to compare the MEDIAGNOST test to commercially available tests were performed by Ranke et. al.[28]. One study evaluated from 427 subjects ages 18 to 74 years. The samples were run in duplicate on the Mediagnost test and two other commercially available RIA to determine the concentration of IGF-I in the samples. The correlation formula to the commercially available assays are: y=1.3x-45 (R= 0.79) and y = 1.4x-26 (R=0.74).

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SUMMARY OF THE ASSAY

Reagent preparation:	**Reconstitution:**	**Dilution:**
1. Antibody (B)	in **11 mL** Assay Buffer **(A)**	
Tracer (C)	in **11 mL** Assay Buffer **(A)**	
NSB (D)	in **500 µL** Assay Buffer **(A)**	
Standards (E-L)	in **500 µL** Dilution Buffer **(DB)**	
Controls (M+N)	in **100 µL** Aqua dest.	**1:101** with **DB**
2.Antibody (O)	in **1 mL** Assay Buffer **(A)** **Mix** immediately before use with **55 mL** Reagent **(P)**	
Dilute **Samples** with Dilution Buffer **(DB)** e.g. **1:101.**		

Assay procedure for double determination

Nr. of Tubes	**Contents of Tubes**	**DB** (Dilution Buffer) **E-L** (Standards) **M,N** (Controls) **Samples**	**D** (NSB)	**B** (1.Antibody)	**C** (Tracer)
1, 2	Total Counts	–	–	–	100
3, 4	NSB	100 **DB**	100	–	100
5, 6	B_0	100 **E**	–	100	100
7 - 20	Standards	100 **F-L**	–	100	100
21, 22	High Control	100 **M**	–	100	100
23, 24	Low Control	100 **N**	–	100	100
25, 26	Sample 1	100	–	100	100
27, 28	Sample 2	100	–	100	100
etc.					
Colour after addition		**Green**		**Blue**	**Violet**

Nr.: 1, 2 remove until counting the activity.
Mix other tubes with a Vortex-Mixer.
Incubation at **35.6-46.4°F (2-8°C), 2 days**
Add **500 µL P** (after addition of reagent **O**) **in all Tubes** The reagent-mix should be cold **35.6-46.4°F (2-8°C).**
Mix with Vortex-Mixer.
Incubation at **35.6-46.4°F (2-8°C), 1 hr**
Add 1 mL ice-cold distilled water
Centrifugation at **3000 x g, 30 min**, **35.6-46.4°F (2-8°C)**
Aspirate the supernatant (as a precaution, e.g. leave approx. 2 mm as a remaining supernatant above the precipitate).
Count the **activity** of all tubes with a Gamma Counter.