IGF-I RIA-CT

Radioimmunoassay with Coated Tubes for the Quantitative Determination of

Insulin-like Growth Factor-I

(IGFBP blocked)

Product Code: IGF-R22

100 tubes

CE

DE/CA40/00809/5
European Union*:
United States of America**:
for in-vitro diagnostics
IVD for professional use!
Rest of the world: For research use only!

mediagnost®
Gesellschaft für Forschung und Herstellung von Diagnostika GmbH

Aspenhaustr. 25 • D-72770 Reutlingen
Phone : + 49 - (0) 7121 51484-0 • Fax: + 49 - (0) 7121 51484 10
E-mail: contact@mediagnost.de • http://www.mediagnost.de

TABLE OF CONTENTS

* Europäische Union / European Union: please ask for package inserts in your national language
** United States of America please ask for special package insert

Read entire protocol before use!

FEATURES

- High Specificity for IGF-I
- Correct measurement of IGF-I in non-extracted samples
- Elimination of interference by IGF-binding proteins through excess IGF-II
- No physical separation of IGF-I from IGF-binding proteins required
- Easy handling:
  - Standards ready for use
  - Separation without centrifugation by means of specifically coated tubes
  - More reliable performance by means of coloured solutions
- 100% recovery of IGF-I leads to correct absolute values
- Small sample volume requirement, thus ideal for young patients

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.

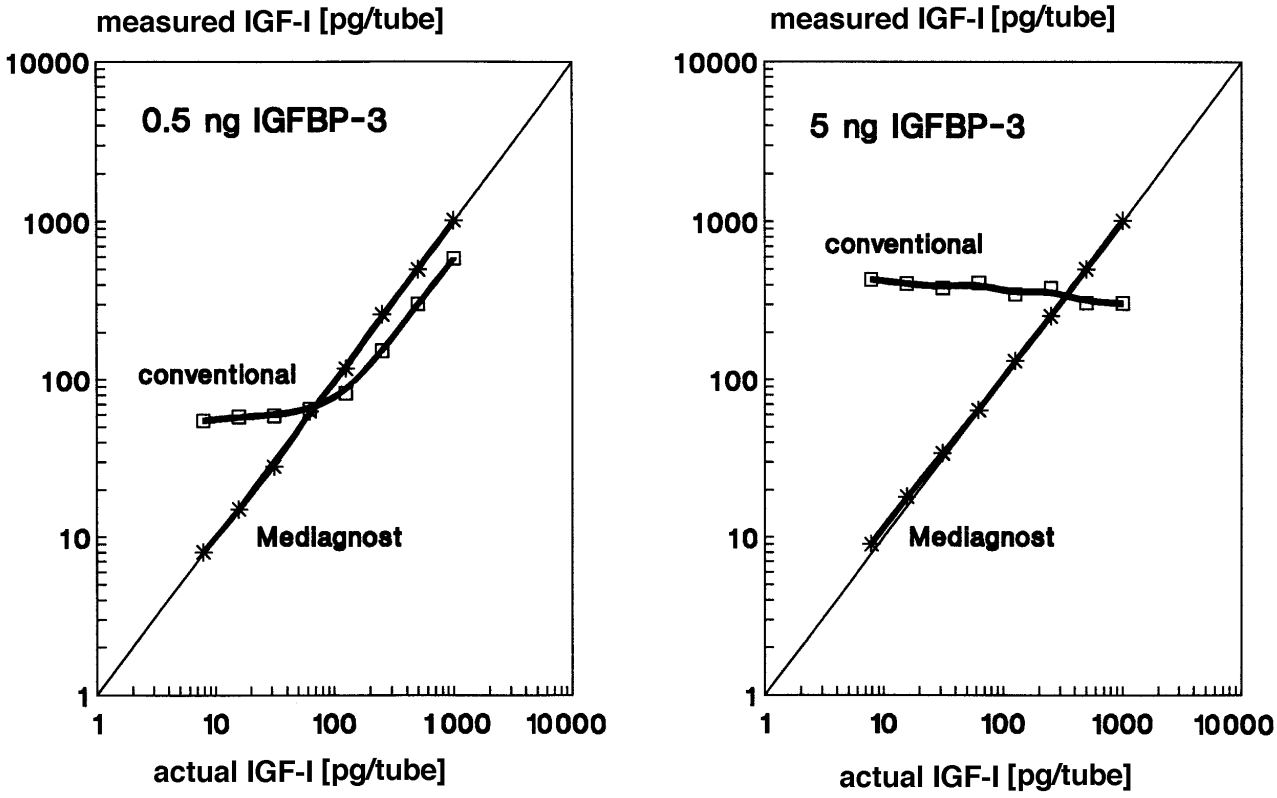

Figure 1. Interference of IGFBP in IGF-I measurements. Known concentrations of IGF-I were assayed in the presence of 0.5 ng (left) or 5 ng (right) hIGFBP-3 by a conventional (□) and by the IGFBP-blocked RIA (*).

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-reproducable 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 SIGNIFIGANCE

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. However, the determination of IGF-I in these specimens is particularly difficult due to the presence of IGFBPs usually in excessive amounts. This explains why conventional assays, in which IGFBPs are not removed, result in incorrect IGF-I values, which reflect more the present amount of IGFBP 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 IGFBP-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 streptavidin-coated 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 measure-ment of free IGF-I. With this method, the IGFBPs are not removed, but their function and therefore their inter-ference 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.

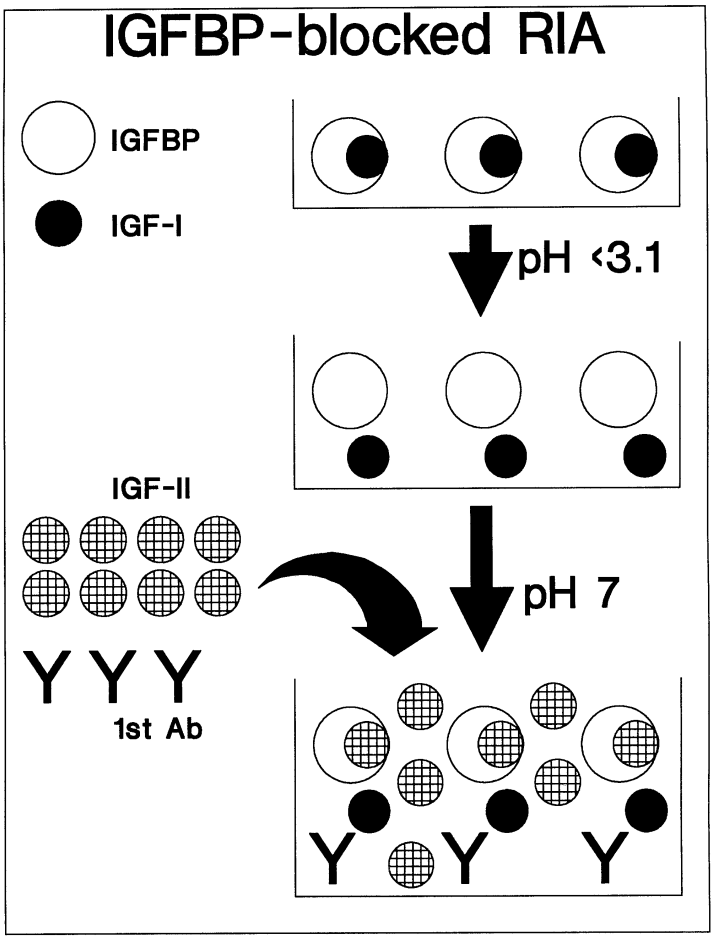

Figure 2. Principle of the IGFBP-blocked IGF-I RIA.

In order to separate bound and free tracer, the immuno-complex (anti- gen-spec. antibody) binds to the capture antibody, that binds in turn to the streptavidin-coated tubes. Therefore, time consuming centrifugations and separations be-come unnecessary. The colour of the solutions makes possible for every tube a control of the respective performance step. This enables you to check your pipetting plan, if necessary.
Standards,dilution and acidification buffer (and by that, the 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 entire incubation colour **violet**.

INTENDED USE

This radioimmunoassay kit is suitable for the scientific and diagnostic measurement of IGF-I in human serum or plasma, or other human body fluids (e.g. follicular fluid, seminal plasma). Due to the high cross-reactivity of the specific 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, mouse, cat, chicken, guinea pig, goat, horse, donkey and dog.

PRECAUTIONS

General

All reagents are for in vitro 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 Sodium-Azide as preservative, however, highly diluted (0.02%). Sodium-Azide is very toxic, R-Phrases: 28, 32, 50/53 and S-Phrases 28, 45, 60, 61 must be considered.

First aid procedures:

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.

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 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 air born, 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 approx. 0.1 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 approx. 3 ng/ml. The inter-assay variation coefficient at 50 % B/B_0 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 plasma samples must be considerably diluted before measurement. No extraction step is required as in conventional IGF-I assays.
This radioimmunoassays is calibrated against the WHO International Reference Standard preparation of IGF-I, **WHO NIBSC 02/254** (25-26).

Clinical Validation

Clinical validation was achived 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.

SAMPLE PREPARATION AND STORAGE

The stability of IGFBP-bound IGF-I makes sample preparation simple. 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 -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 cyles. 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.

MATERIALS

Materials provided

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

DB Dilution Buffer
(1 bottle, 125 ml, ready for use, coloured)

A Assay Buffer
(2 bottles, 1 x 60 ml + 1 x 30 ml, ready for use)

R Capture Antibody: anti-rabbit-IgG, biotin-conjugated
(1 bottle, 5.5 ml, lyophilized)

S Specific Antibody: rabbit-anti-hIGF-I (containing recomb. hIGF-II), (1 bottle, 5.5 ml, lyophilized)

C Tracer (125I-IGF-I) (< 1.5 µCi or < 55 kBq)
(1 bottle, 11 ml, lyophilized, red colured)

F -J Standards. (Concentrations given on vial-labels in ng/ml)
(5 vials, 750 µl each, ready for use)

M+N Controls (Concentrations given on vial-labels in ng/ml)
(2 vials, 100 µl each, human serum, lyophilized)

T Tubes
(100 tubes, coated with streptavidin)

Required Materials Not Provided

1) Pipettes: 10 ml, 500 µl, 250 µl, 100 µl, 10 µl, 25 µl, 100 µl, and 250 µl repeating pipettes are recommended.
2) Shaking device
3) Device for aspiration of liquids (e.g. connected to a water pump).
4) Gamma counter

ASSAY PROCEDURE

Reagent Preparation

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

R Reconstitute with **5.5 ml** reagent **A (Assay Buffer)**.

S Reconstitute with **5.5 ml** reagent **A (Assay Buffer)**.

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

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

Further dilution according to sample dilution with reagent **DB (Dilution Buffer)** (e.g. 1:26)

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

Normally serum or plasma samples have to be diluted with **Dilution Buffer DB**. This dilution with **Dilution Buffer DB** simultaneously leads to the necessary acidification to pH≤3 of the sample. For routine applications we recommend a dilution of 1:26.
Example: Add 10 µl sample to 250 µl **Dilution Buffer DB** (dilution factor 26).

The dilution of the **controls M & N** with **Dilution Buffer DB** should be according to the common dilution of serum or plasma samples, e.g. 1:26.

For samples as expected **with extremely low IGF-I contents**, non serum or plasma samples (e.g. Saliva or urine) the more sensitive **Mediagnost RIA IGF-R20** might be better suited.

Procedure

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

Flow Chart of Assay Protocol:

Nr.	Tubes	DB F-N Samples	S	R	C
1,2	TC	---	---	---	100
3,4	B_0	**DB**: 100	50	50	100
5-14	Standards	**F-J**: 100	50	50	100
15,16	High Control	**M**: 100	50	50	100
17,18	Low Control	**N**: 100	50	50	100
19,20	Sample 1	100	50	50	100
21,22	Sample 2	100	50	50	100
etc.					
Colour :		**Green**	After addition: **Blue**		After addition **Violet**

(All volumes are given as µl.)

1) Labelling of the assay tubes should be done in the following order:

1, 2 total counts (**TC**)
3, 4 **Dilution Buffer DB** (zero standard, **B_0**)
5 - 14 duplicates of **Standards (F to J)**,
15, 16 duplicates of **High Control (M)**,
17, 18 duplicates of **Low Control (N)**,
19, 20 etc. duplicates of **samples**

2) Add **100 µl** of **Dilution Buffer DB** to tubes 3 - 4.

3) Add **100 µl** of reagents **F - J (Standards)** to tubes 5 - 14:

5, 6 Standard F
7, 8 Standard G, etc.

4) Add **100 µl** of **diluted reagent M (High Control)** to tubes 15 and 16.

5) Add **100 µl** of **diluted reagent N (Low Control)** to tubes 17 and 18.

6) Add **100 µl** of diluted sample to tubes 19 and 20,etc.

-All solutions appear **green**!-

7) Add **50 µl** reagent **S (Spec. Antibody)** beginning with tube 3.

-All solutions turn **blue**!-

8) Add **50 µl** reagent **R (Capture-Antibody)** beginning with tube 3.

9) Add **100 µl** reagent **C (tracer)** to all tubes.

-All solutions turn **violet**!-

Remove tubes 1 and 2 (total counts, **TC**) or seal with a stopper.

10) **Shake** the tubes on a shaking device **(350 rpm) at least 4 h** at **room temperature**.

Alternative Incubation:

A) Over night: (>15h), 350 rpm, RT
B) Over night: (>15h), without shaking, 2-8°C
(In this case contents of the tubes must be initially mixed by unique shaking or vortexing)
C) Extented Incubation: For example over the weekend (e.g. 60 h) is possible with the version B

11) Aspirate the liquid (except tubes 1 and 2 !) completely.

Take care that the coating of the tubes remains intact. Depending on laboratory equipment and common laboratory practice, aspiration of the liquid can be replaced by careful decantation.

12) Add **500 µl** of reagent **A (Assay Buffer)** to the tubes (except tubes 1 and 2 !).

13) Aspirate the liquid (see step 11).

14) Count the radioactivity of **all** tubes.

Alternative Pipetting Schema for working steps 7 and 8

Mix the reconstituted **Reagents S** (**Spec. Antibody) and R (Capture Antibody)** externally (1:1), add 100 µl of this mix beginning with the tube 3.

EVALUATION OF RESULTS

Establishing of the Standard Curve

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

Standard	**F**	**G**	**H**	**I**	**J**
ng/ml	0.37	1.11	3.33	10	30

1. Calculate the average counts of each pair of tubes. This equals to the binding value B.
2. The mean value of the Dilution Buffer (DB: tubes 3 and 4) equals B_0.
3. Calculate the percent bound (% B/B_0):
   $\%B/B_0 = B/B_0 \times 100\%$.
4. Plot % B/B_0 versus the standard concentrations on a semi-logarithmic or logit-log paper respectively. For convenience, it is recommended to use computer assisted data reduction programs.
5. Calculate the ‘percentage bound of the zero standard‘:
   B_0 / total counts TC x 100%
   = (average of tubes 3, 4 / average of tubes 1, 2) x 100%.
   It should be $\%B_0/TC > 25\%$.

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 zero standard (B_0): 9005 cpm

average counts of sample: 3812 cpm

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

= (3812) / (9005) x 100%

= 0.423 x 100%

= 42.3%

For a 42.3 % value on the y-axis (ordinate) a value of 5.92 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 (e.g.: 26).

Example: 5.92 x 26 = 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 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

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 and19 years of age were classified according to gender, as the pubertal peak occures 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) (27).

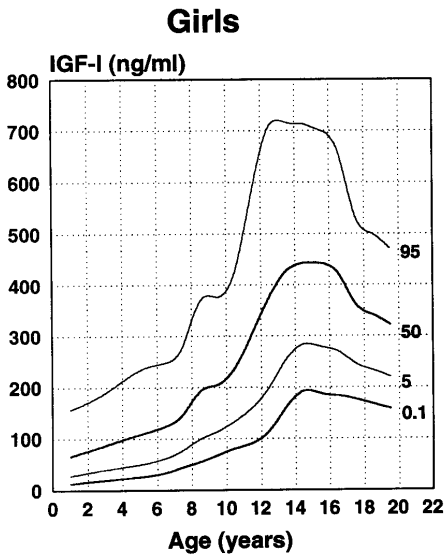

Fig. 3: Age-dependent normalrange of serum IGF-I levels in girls

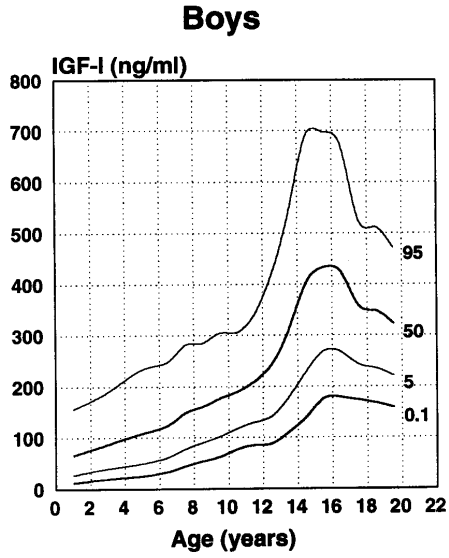

Fig. 4: Age-dependent normal range of serum IGF-I levels in boys

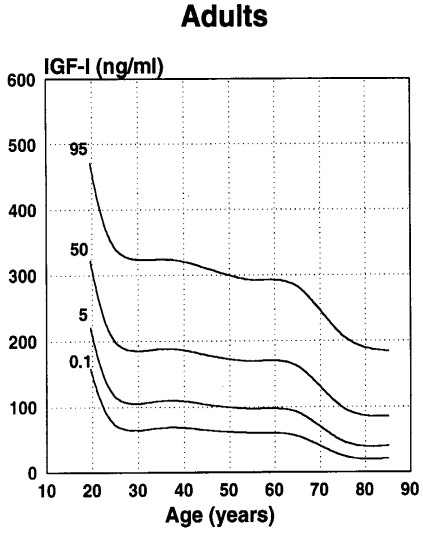

Fig. 5: Age-dependant normal range of serum IGF-I levels in adults

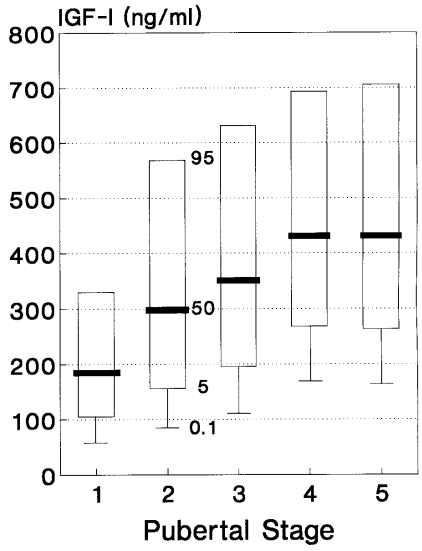

Fig 6.: Serum IGF-I levels in normal children and adolescents (7 to 17 years) according to pupertal stages. Both sexes were included.

PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical sensitivity of the radioimmunoassay for IGF-I R22 yields 0.107 ng/ml 2x SD of zero standards in 15-fold determination.

Specificity

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

	IGF-II	Insulin	Proinsulin
Reactivity [%]	0.021	0.061	0.024

Reproducibility

Intra-Assay-Variation

	Number of determinations	Mean value (ng/ml)	Standard deviation (ng/ml)	CV (%)
Sample 1	8	179.6	4.5	2.5
Sample 2	8	55.8	2.6	4.6
Sample 3	8	140	4.7	3.4

Inter-Assay-Variation

	Number of determinations	Mean value (ng/ml)	Standard deviation (ng/ml)	CV (%)
Sample 1	18	186.1	8.31	4.5
Sample 2	18	54.8	2.71	4.9
Sample 3	18	137.9	8.61	6.2

Linearity

Dilution:	Sample 1 (calculated, ng/ml)	Dilution:	Sample 2 (calculated, ng/ml)
1:20	175.9	1:20	126.4
1:23	175.6	1:22	130.2
1:26	172.8	1:26	137.6
1:30	183.6	1:30	133.8
1:35	186.0	1:35	143.6
1:40	188.2	1:40	136.0
AV / 1SD	180.9 / 6.04	AV / 1SD	134.6/ 5.99

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 102.5% % of the hypothetical expected amount.

100ng/ml IGF-I added	Serum 1	Serum 2
Recovery [%]	105.9%	98.2%

REFERENCES

1) Baxter RC. 1986 The somatomedins:insulin-like growth factors. Adv Clin Chem.25:49-115

2) Daughaday WH, Rotwein P. 1989 Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. Endocr Rev. 10:68-91

3) Spencer EM (Ed.) 1991 Modern Concepts of Insulin-Like Growth Factors. New York: Elsevier.

4) Klapper DG, Svoboda ME, Van Wyk JJ. 1983 Sequence analysis of somatomedin-C: confirmation of identity with insulin-like growth factor-I. Endocrinology. 112:2215-2217.

5) Rinderknecht E, Humbel RE. 1978 The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. J Biol Chem. 253:2769-2276.

6) Clemmons DR, Van Wyk JJ. 1984 Factors controlling blood concentration of somatomedin C. Clin Endocrinol Metab. 13:113-143.

7) Ballard J, Baxter R, Binoux M, et al. 1989 On the nomenclature of the IGF binding proteins. Acta Endocrinol (Copenh). 121:751-752.

8) Drop SLS. 1992 Report on the nomenclature of the IGF binding pro-teins. J. Clin Endocrinol Metab. 74:1215-1216.

9) Martin JL, Baxter RC. 1986 Insulin-like growth factor binding protein from human plasma. Purification and characterization. J Biol Chem. 261:8754-8760.

10) Binkert C, Landwehr J, Mary JL, Schwander J, Heinrich G. 1989. Cloning, sequence analysis and expression of a cDNA encoding a novel insulin-like growth factor binding protein (IGFBP-2). EMBO J. 8:2497-2502.

11) Furlanetto RW, Underwood LE, Van Wyk JJ, D'Ercole AJ. 1977. Estimation of somatomedin-C levels in normals and patients with pituitary disease by radioimmunoassay.J. Clin Invest. 60:648-657.

12) Daughaday WH, Kapadia M, Mariz I. 1987 Serum somatomedin binding proteins: physiologic significance and interference in radioligand assay. J Lab Clin Med. 109:355-363.

13) Breier BH, Gallaher BW, Gluckman PD. 1991 Radioimmunoassay for insulin-like growth factor-I: solutions to some potential problems and pitfalls. J Endocrinol. 128:347-357.

14) Daughaday WH, Mariz IK, Blethen SL. 1980 Inhibition of access of bound somatomedin to membrane receptor and immunobinding sites: a comparison of radioreceptor and radioimmunoassay of somatomedin in native and acid-ethanol-extracted serum. J Clin Endocrinol Metab. 51:781-788.

15) Blum WF, Gallaher B, Ranke MB. 1992 An IGFBP-blocked IGF-I RIA that measures what it pretends to measure: IGF-I. 74th Annual Meeting of the American Endocrine Society. 293.

16) Rosenfeld RG, Wilson DM, Lee PDK, Hintz RL. 1986 Insulin-like growth factors I and II in evaluation of growth retardation. J Pediatr. 109:428-433.

17) Clemmons DR, Van-Wyk JJ, Ridgway EC, Kliman B, Kjellberg RN, Underwood LE. 1979 Evaluation of acromegaly by radioimmuno-assay of somatomedin-C.N.Engl J Med. 301:1138-1142

18) Zapf J, Walter H, Froesch ER. 1981 Radioimmunological determination of insulin-like growth factors I and II in normal subjects and in patients with growth disorders and extrapancreatic tumor hypoglycemia. J Clin Invest. 68:1321-1330.

19) Blum WF. 1992 Insulin-like growth factors and their binding proteins. In: Ranke MB, ed. Functional Endocrinologic Diagnostics in Children and Adolescence. Mannheim: J + J Verlag; 102-117.

20) Rieu M, Girard F, Bricaire H, Binoux M. 1982 The importance of insulin-like growth factor (somatomedin) measurements in the diagnosis and surveillance of acromegaly. J Clin Endocrinol Metab. 55:147-153.

21) Blum WF, Ranke MB, Bierich JR. 1988 A specific radioimmunoassay for insulin-like growth factor II: the nterference of IGF binding proteins can be blocked by excess IGF-I. Acta Endocrinol (Copenh).118:374-380.

22) Wilson EM, Oh Y, Rosenfeld RG (1997) Generation and characterization of an IGFBP-7 antibody: Identification of 31 kD IGFBP-7 in human biological fluids and Hs578T human breast cancer conditioned media. J Clin Endocrinol Metab Vol 82, 4:1301-1303.

23) Ranke MB, Schweizer R, Elmlinger MW, Weber K, Binder G, Schwarze CP, Wollmann HA (2000) Significance of basal IGF-I, IGFBP-3 and IGFBP-2 measurements in the diagnostics of short stature in children. Horm Res 54:60-68

24) Ranke MB, Schweizer R, Elmlinger MW, Weber K, Binder G, Schwarze CP, Wollmann HA (2001) Relevance of IGF-I, IGFBP-3 and IGFBP-2 measurements during GH treatment of GH-deficient and non-GH-deficient children and adolescents. Horm Res 55:155-124

25) Address NIBSC:Blance Lane, South Mimms, Potters Bar, Hertford EN 6 3 QG, Great Britain

26) Burns C, Rigsby P, Moore M, Rafferty B. (2009) The first International Standard for Insulin-like Growth Factor-1 (IGF-1) for immunoassay: Preparation and calibration in an international collaborative study. Growth Hormone & IGF Research (2009)

27) Blum WH, Breier BH (1994) Radioimmunoassays for IGFs and IGFBPs. Growth Regulation 4 (Suppl. 1):11-19

SUMMARY OF THE ASSAY

Reagent Preparation:	Reconstitution:	Dilution:
Capture Antibody (R)	in **5.5 ml** Assay Buffer **(A)**	
Specific Antibody (S)	in **5.5 ml** Assay Buffer **(A)**	
Tracer (C)	in **11 ml** Assay Buffer **(A)**	
Control (M)+(N)	in **100 µl** A.dest. each	**1:26** with **DB**
Dilute **Plasma/Serum Samples** with Dilution Buffer **(DB)** e.g. **1:26**. If very low IGF-I levels are expected, see page 14.		

Assay Procedure for Double Determinations

Addition of Reagent [µl]					
Nr. of Tubes	**Contents of Tubes**	**DB F-N Samples**	**S**	**R**	**C**
1,2	Total Counts (TC)	–	–	–	100
3,4	B_0	100 **DB**	50	50	100
5-14	Standards	100 **F-J**	50	50	100
15,16	High Control **M**	100 **M**	50	50	100
17,18	Low Control **N**	100 **N**	50	50	100
19,20	Sample 1	100	50	50	100
21,22 (etc)	Sample 2 (etc)	100	50	50	100
Colour:		**green**	After addition: **blue**		After addition: **violet**

Tubes Nr.:1,2 remove until counting the activity
Incubation, at least. **4 hours**, at **RT, 350 rpm** (Alternatively: Incubate **over night** without shaking (i.e., at least **15 hours**) at **2 - 8°C,** mix tubes before incubation see page 16)
Aspirate the liquid completely. Take care that the coating of the tubes remains intact.
Add **500 µl** of reagent **A (Assay Buffer)** to the tubes
Aspirate the liquid completely (see above)
Count the radioactivity of all tubes.