MEASUREMENT OF IGFBP-3 CONCENTRATIONS IN HUMAN SERUM

Assay Principle:

IGFBP-3 levels are determined using the "Active" IGFBP-3 IRMA kit manufactured by Diagnostic Systems Laboratories, Inc. (DSL, Webster, Texas). The calculated sensitivity of the kit is 0.5 ng/ml. The kit employs a two-site immunoradiometric principle to directly measure non-glycosylated IGFBP-3. In this non-competitive assay, the analyte is "sandwiched" between two antibodies. One antibody is immobilized on a solid carrier (the tube) while the second is labeled with the [^{125}I] tracer. The unbound reagents are removed by decanting and washing the tubes. The activity of the "sandwiched" complex is measured to determine the IGFBP-3 concentration.

Assay Procedure:

Frozen specimens are thawed and prepared for analysis by diluting 10μl of serum with 1 ml IGFBP-3 Sample Diluent.

An in-house human control (QCV) is diluted (10μl serum plus 1 ml Diluent) and analyzed simultaneously to verify the effectiveness of the assay.

Human control samples provided with the kit (Levels I and II) are not diluted prior to assay but are analyzed simultaneously.

Creation of the assay standard curve is achieved using undiluted standards (B-F) containing known concentrations of IGFBP-3 in a protein-based buffer.

Controls, standards and diluted unknowns (50μl) are pipetted into the bottom of tubes coated with anti IGFBP-3.

[^{125}I] anti IGFBP-3 antibody (200μl) is then added to all tubes.

The mixture is allowed to incubate at room temperature (~25°C) for 18-24 hours on a shaker set at 180 rpm.

MEASUREMENT OF IGFBP-3 CONCENTRATIONS IN HUMAN SERUM

Assay Procedure (continued):

Unbound radiolabeled anti IGFBP-3 is removed by decanting and washing three times with 3 ml deionized water.

The bound radioactivity is determined using a Packard RIAStar Gamma Counter model C5410.

Concentrations of IGFBP-3 are determined by comparing the resultant cpm of the unknowns and controls to the standard curve constructed for known IGFBP-3 standards. Sample results are automatically corrected for the dilution factor.

The IGFBP-3 data is transferred to a Power Macintosh 6500/250 and set-up as a Microsoft Excel spreadsheet.

ACTIVE™ IGFBP-3 IRMA

DSL-6600

Revision date: January 21, 1997

Diagnostic Systems Laboratories, Inc.
Corporate Headquarters, 445 Medical Center Blvd.
Webster, Texas 77598-4217 USA
General Business: Tel: 281.332.9678
Customer Assistance Center: Tel: 800.231.7970 Fax: 281.338.1895
e-mail: mktg@dslabs.com
Website: http://www.dslabs.com

. INTENDED USE

he DSL-6600 ACTIVE™ Insulin-like Growth Factor Binding Protein-3 (IGFBP-3) Coated-Tube mmunoradiometric Assay Kit provides materials for the quantitative measurement of IGFBP-3 in erum. This assay is intended for *in vitro* diagnostic use. Measurement of serum IGFBP-3 is sed as a diagnostic aid in the evaluation of growth hormone-related disorders.

. SUMMARY AND EXPLANATION OF THE TEST

he insulin-like growth factors, IGF-I and IGF-II, are potent mitogenic and anabolic peptides ith structural homology to insulin [1]. The IGFs are produced by multiple body tissues under ontrol of growth hormone (GH) and other regulatory factors, and act as autocrine, paracrine nd endocrine regulators of cell growth. The actions of the IGFs and insulin are mediated by inding to cell surface receptors [2], including the type 1 IGF (IGF-I), type 2 IGF (IGF-II) eceptors and the highly homologous insulin receptor.

n serum and other biological fluids, the IGFs are complexed to specific, structurally homologous inding proteins (IGFBPs) [3-5]. Six IGFBPs have been cloned and sequenced in both human nd rat [4]. The IGFBPs are numbered according to the sequence of their molecular haracterization [6].

luman IGFBP-3 is a 264 amino acid peptide, with a 27 amino acid signal peptide and 18 ysteine residues [7]. The predicted MW of the nonglycosylated protein is 29 KDa, which orresponds to the M_r of expressed nonglycosylated protein [9]. On reducing gels, the peptide igrates with an apparent M_r of 29K, while glycosylated forms migrate at ~48K and ~44K [9].

GFBP-3 is the major IGFBP in postnatal serum, where its molar concentration in normal serum pproximates the molar concentration of total IGF peptide (IGF-I + IGF-II) [6]. Efforts to alculate serum IGFBP-3 concentrations by competitive binding studies or charcoal precipitation ave been hampered by the presence of other IGFBPs in serum and other biological fluids [3-5]. he development of the western ligand blotting technique by Hossenlopp et al. [10] permits iscrimination of IGFBP-3 from other IGFBPs, especially when combined with deglycosylation udies and/or immunoprecipitation [3]. However, it has been shown recently that IGFBP-3 rotease are present in multiple biological fluids, most notably pregnancy serum [11,12], mniotic fluid [13], prostatic fluid [14], and urine [15]. IGFBP-3 is also degraded by plasmin 6]. Proteolyzed IGFBP-3 is often undetectable by conventional ligand blotting methodologies 1-16].

he recent development of specific radioimmunoassays for IGFBP-3 has permitted the accurate uantification of IGFBP-3 concentrations in serum. Serum levels rise gradually during ildhood, increase more rapidly during puberty, reach peak levels at mid to late puberty, and ll during adult life [17].

etermination of serum IGFBP-3 levels promises to be of considerable value in assessment of sorders of the GH-IGF axis. Like IGF-I, IGFBP-3 is GH dependent; however, IGFBP-3 levels ay be less age-dependent than IGF-I levels [17]. Total IGF levels show higher correlation with H sufficiency than either IGF-I or IGF-II levels alone [18] and IGFBP-3 levels correspond with tal IGF levels. In addition, IGF assays typically require an extraction procedure to remove terfering IGF-binding proteins; this step may be a source of assay error [19]. Assays for GFBP-3 do not require prior sample extraction.

ese aspects of IGFBP-3 physiology and chemistry allow its measurement to reflect GH activity. us, in GH deficiency of either pituitary or hypothalamic origin, IGFBP-3 levels are uniformly w. Blum et al [17] have reported that 128/132 children with GH deficiency have serum GFBP-3 concentrations below the 5th percentile for age. Similar data have been reported by segawa et al. [20]. In situations of GH receptor deficiency, such as Laron syndrome, anti-GH tibodies, liver disease or malnutrition, IGFBP-3 levels are also reduced, even in the presence elevated serum GH concentrations [21].

. PRINCIPLE OF THE TEST

e procedure employs a two site immunoradiometric assay (IRMA) principle described by Miles al. [22]. The IRMA is a non-competitive assay in which the analyte to be measured is andwiched" between two antibodies. The first antibody is immobilized to the inside walls of the bes. The other antibody is radiolabelled for detection. The analyte present is bound by both the antibodies to form a "sandwich" complex. Unbound reagents are removed by decanting d washing the tubes.

. REAGENTS

e DSL-6600 ACTIVE™ IGFBP-3 Coated-Tube IRMA Kit contains sufficient reagents for 100 bes. Each kit contains the following reagents:

IGFBP-3 Standard A/Sample Diluent:
ne bottle, 55 mL, labeled A, containing 0 ng/mL IGFBP-3 in a protein-based (BSA) buffer with dium azide as a preservative. Store at 2-8°C until expiration date. Do not use after piration.

B. IGFBP-3 Standards: (Lyophilized)
Five vials, labelled B-F, containing concentrations of approximately 2.0, 5.0, 20.0, 50.0, 100.0 ng/mL of IGFBP-3 in a protein-based (BSA) buffer. Refer to vial labels for exact concentrations. Reconstitute each vial with 1 mL deionized water. Store unopened at 2-8°C until expiration date. After reconstitution, Standards may be stored at 2-8 °C for up to 48 hours or at -20°C or lower for up to two weeks. Longer storage periods are not recommended.

NOTE: The DSL-6600 IGFBP-3 IRMA Standards and Controls are calibrated to recombinant DNA-derived non-glycosylated human IGFBP-3 (MW 28.75 kDa) expressed by E. coli obtained from Celtrix Pharmaceuticals, Inc. The original reference preparation was quantified by amino acid analysis.

C. Anti-IGFBP-3 [I-125] Reagent: (YELLOW)
One bottle, 22 mL, containing < 10 µCi (370 kBq) of [I-125]-labelled goat Anti-IGFBP-3 polyclonal antibody in a protein-based (BSA) buffer with sodium azide as a preservative. Store at 2-8°C until expiration date.

D. Anti-IGFBP-3-Coated Tubes: (CLEAR WITH GREEN RIM)
Four bags, each containing 25 plastic tubes with goat anti-IGFBP-3 polyclonal immunoglobulin immobilized to the inside wall of each tube. Store the tubes at 2-8°C until expiration date only in the bags in which they are supplied.

NOTE: *The process which is used to manufacture the antibody-coated tubes sometimes causes a crystalline substance to form on the inside walls of the tubes. This will not affect the performance of the assay in any way.*

E. IGFBP-3 Controls: (Lyophilized)
Two vials, Levels I and II, containing low and high levels of IGFBP-3 in a protein-based (BSA) buffer with sodium azide as a preservative. Reconstitute each vial with 1 mL deionized water. See vial labels for ranges. Store unopened at 2-8°C until expiration date. Upon reconstitution, store at 2-8 °C for up to 48 hours or at -20 °C or lower for up to two weeks. Longer storage periods are not recommended.

V. PRECAUTIONS

For *in vitro* diagnostic use.

Radioactive Material -- Not for Internal or External Use in Humans or Animals.

This radioactive material may be received, acquired, possessed, and used only by physicians, clinical laboratories or hospitals and only for *in vitro* clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations of each country.

The following precautions should be observed in handling radioactive material:

- Store radioactive materials in a designated area.
- Do not eat, drink, smoke or apply cosmetics where radioactive materials are being handled.
- Do not pipet by mouth.
- Wear gloves when handling radioactive materials and wash hands thoroughly afterwards.
- Cover working area with disposable absorbent paper.
- Wipe up all spills immediately and thoroughly and dispose of the contaminated materials as radioactive waste.
- Dispose of the liquid radioactive waste into the sanitary sewage system if permitted by the local regulations.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL
This kit may contain reagents made with human serum or plasma. The serum or plasma used has been tested by an FDA-approved method and found to be non-reactive for HIV-1/2 Antibodies, HCV and HBsAg. Because no method can offer complete assurance that HIV-1/2, HCV, HBsAg or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 3rd Edition 1993.

CHEMICAL HAZARD:
Some reagents in this kit contain sodium azide as a preservative. For all such reagents, the concentration of sodium azide is ≤ 0.09%. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Dispose of all nonradioactive reagents by flushing with large amounts of water through the plumbing system.

VI. SPECIMEN COLLECTION AND PREPARATION

Serum should be used and the usual precautions for venipuncture should be observed. Undiluted samples may be stored at 2-8 °C for up to 48 hours or at -20 °C or lower for at least 8 weeks. Samples which have been diluted for the assay (see Assay Procedure) may be stored at 2-8 °C for up to 24 hours or at -20 °C or lower for up to 4 weeks. Use of heparinized or EDTA plasma is not recommended.

Typical serum samples should be diluted 1:100 with the IGFBP-3 Sample Diluent (0 ng/mL Standard A) prior to assay.

VII. PROCEDURAL NOTES

A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the instructions in the package insert. A standard curve must be included with each assay.

Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label.

After removing assay reagents from the refrigerator, allow them to reach room temperature (~25°C) before pipetting. Unused reagents should be stored as indicated in the Reagents Section. Standards and Controls should be mixed before use by inverting or swirling gently rather than vortexing. To insure a homogenous mixture of the reagents in each assay tube, gentle and thorough shaking or vortexing is essential.

Inadequate decanting of tubes and/or failure to blot tubes following decantation may result in poor replication and spurious values.

VIII. TEST PROCEDURE

A. Materials Supplied:

Materials supplied in the DSL ACTIVE™ IGFBP-3 Coated-Tube IRMA Kit, Catalog No. DSL-6600:

MATERIAL	QUANTITY	CATALOG NO.
IGFBP-3 Sample Diluent (Standard A)	One Bottle	6601
IGFBP-3 Standard B	One Vial	6602
IGFBP-3 Standard C	One Vial	6603
IGFBP-3 Standard D	One Vial	6604
IGFBP-3 Standard E	One Vial	6605
IGFBP-3 Standard F	One Vial	6606
Anti-IGFBP-3-Coated Tubes	Four Bags	6610
Anti-IGFBP-3 [I-125] Reagent	One Bottle	6620
IGFBP-3 Control Level I	One Vial	6651
IGFBP-3 Control Level II	One Vial	6652

B. Materials Required But Not Supplied:

- 12 x 75 mm polypropylene test tubes.
- Test tube rack for 12 x 75 mm tubes.
- Precision pipette to deliver 50 µL.
- Precision repeating pipette to deliver 200 µL and 3 mL.
- Vortex mixer.
- Sponge test tube rack or similar device for decantation.
- Absorbent material for blotting tubes.
- Gamma counter.
- Log-log graph paper or computer IRMA data analysis program.

C. Assay Procedure:

Allow all reagents to reach room temperature (~25°C) and mix thoroughly before use. After reconstitution of Standards and Controls, mix thoroughly, avoiding foam. Assay Standards, Controls and unknowns in duplicate.

1. Human Serum samples should be diluted 1:100 with IGFBP-3 Sample Diluent prior to assay. (Example: 10 µL serum + 1 mL IGFBP-3 Sample Diluent). Samples with very low expected values should be diluted 1:50 with IGFBP-3 Sample Diluent prior to assay. (Example: 10 µL serum + 500 µL IGFBP-3 Sample Diluent)
   Note: Controls should not be diluted prior to assay and results for the Controls should not be multiplied by sample dilution factor.
2. Label two plain (uncoated) tubes for Total Count tubes. Label and arrange coated tubes in duplicate for Standards, Controls and unknowns. Reseal the bags of unused tubes carefully and store them at 2-8°C.
3. Add 50 µL of the Standards, Controls or unknowns to the appropriate tubes.
4. Add 200 µL of the Anti-IGFBP-3 [I-125] Reagent to all tubes.
5. Vortex all tubes gently.
6. Incubate all tubes at room temperature (~25°C) overnight (18-24 hours) on a shaker set at 180 rpm.
7. Decant all tubes, except Total Count tubes, by simultaneous inversion with a sponge rack into a radioactive waste receptacle. Strike the tubes sharply on absorbent material to facilitate complete drainage and then blot the tubes for 1-2 minutes to remove any droplets adhering to the rim before returning them to the upright position. Failure to blot tubes adequately may result in poor replication and spurious values.
8. Wash all tubes, except Total Count tubes, by adding 3 mL of deionized water.
9. Decant per Step 7 and repeat wash step two times for a total of three washings.
10. Count all tubes in a gamma counter for one minute.
11. Multiply sample results by dilution factor.

IX. RESULTS

The results in this package insert were calculated using a log-log curve fit. Other data reduction methods may give slightly different results.

Results are expressed as ng/mL. Results may be converted to nmol/L using the following formula, which is based on the molecular weight of the non-glycosylated reference standard (28.75 kDa):

$$\text{ng/mL} \times 0.035 = \text{nmol/L}$$

- Calculate the net counts per minute (net CPM) by subtracting the mean counts per minute (mean CPM) of the 0 ng/mL Standard from the mean CPM of each Standard, Control and unknown. Calculate the % B/T for each Standard Control and unknown as follows:

$$\%\ B/T = \frac{\text{Net CPM}}{\text{Mean Total CPM}} \times 100$$

- Plot a curve of the net CPM or % B/T for each Standard (y-axis) against the IGFBP-3 concentration (x-axis) on log-log graph paper. Draw a standard curve through the mean of the duplicate points.
- Based on the net CPM or % B/T for each Control and unknown, determine the IGFBP-3 concentration of the means of the duplicate counts of each Control and unknown from the standard curve.
- Multiply sample results by dilution factor. Control results should not be multiplied by sample dilution factor.
- After correction, serum values will typically be on the order of 1000 ng/mL. To convert corrected patient samples to mg/L:

$$1000\ \text{ng/mL} = 1\ \text{mg/L}$$

- This assay exhibits linearity from approximately 2.0 - 115.0 ng/mL of IGFBP-3. Any sample with specific cpm greater than the highest Standard should be appropriately diluted with the IGFBP-3 Sample Diluent and reassayed. The level of the highest Standard is approximately 115.0 ng/mL and may vary slightly by lot number; check the vial label for exact concentration.
- Any sample reading lower than the lowest Standard should be reported as such.

X. LIMITATIONS

- The reagents supplied in this kit are optimized to measure IGFBP-3 levels in serum. Use of EDTA or heparinized plasma is not recommended.
- Serum containing IGFBP-3 protease activity may contain immunoreactive IGFBP-3 fragments. Measurement of intact IGFBP-3 in these samples may require preliminary size-exclusion chromatography [21].
- Repeated freezing and thawing of reagents and specimens must be avoided.
- Hemolyzed and lipemic specimens may give false values and should be avoided.
- Any sample reading greater than the highest standard should be diluted appropriately with the IGFBP-3 Sample Diluent and reassayed. The level of the highest Standard is approximately 115.0 ng/mL and may vary slightly by lot number; check vial label for exact concentration.

XI. QUALITY CONTROL

- Low and high level controls should be included with each assay.
- DSL Controls or other commercial controls should fall within established confidence limits. The confidence limits for the DSL Controls are printed on the Control vial labels.
- A complete standard curve and controls should be included with each assay.

TYPICAL STANDARD CURVE FOR ACTIVE™ IGFBP-3 IRMA

TUBE NO.	TUBE LABEL	BOUND (cpm)	MEAN (cpm)	B/T (%)	IGFBP-3 (ng/mL)
1,2	TOTAL COUNTS	153129 151063	152096		
	STANDARDS				
3,4	A	994 997	995	—	0
5,6	B	4773 4479	4626	2.4	2.5
7,8	C	7476 7735	7606	4.3	6.0
9,10	D	23582 22305	22943	14.4	21.0
11,12	E	44876 44634	44755	28.8	50.0
13,14	F	66789 67372	67081	43.5	100.0
	CONTROLS				
15,16	Level I	7582 8211	7897	4.5	6.14
17,18	Level II	22657 21308	21983	13.8	19.41

CAUTION: *The above data must not be employed in lieu of data obtained by the user in the laboratory.*

XII. EXPECTED VALUES

Each laboratory should establish its own expected value ranges. Serum IGFBP-3 levels increase with age during childhood, peak in mid- to late-puberty and decline gradually during adult life. Levels differ between males and females within each age group and pubertal stage. In addition, other factors, such as nutritional status, may affect IGFBP-3 levels. Therefore, it is important that each laboratory carefully define the characteristics of the reference population prior to determining expected values.

The data below are from multi-center studies performed with the DSL-6600 ACTIVE™ IGFBP-3 IRMA kit.

PATHOLOGIC SAMPLES

DIAGNOSIS	AGE (yrs, mean ± SD)	N	MEAN (ng/mL)	SD (ng/mL)	MEDIAN (ng/mL)	ABSOLUTE RANGE (ng/mL)
Growth Hormone Deficiency	8.6 ± 4.6	43	1354	695	1210	250 - 2960
Constitutional Growth Retardation	10.8 ± 3.2	18	2607	719	2530	1520 - 4320
Normal Variant Short Stature	7.3 ± 4.2	20	1994	601	1830	1270 - 2990
Acromegaly	adult	57	5777	1374	5540	1550 - 9370

The pediatric data in the following tables were obtained for children with normal heights and growth patterns.

IGFBP-3 LEVELS BY CHRONOLOGICAL AGE

Male and Female combined:

AGE (years)	N	MEAN (ng/mL)	SD (ng/mL)	MEDIAN (ng/mL)	ABSOLUTE RANGE (ng/mL)
0-1	49	1965	548	1990	1030 - 3090
1-2	42	2154	649	2080	1100 - 3620
2-3	28	2231	605	2140	1200 - 3990
3-4	18	2312	786	2120	1400 - 4250
4-5	23	2363	459	2290	1630 - 3150
5-6	19	2676	553	2720	2000 - 4230
6-7	22	2924	617	2990	2000 - 4210

Male

AGE (years)	N	MEAN (ng/mL)	SD (ng/mL)	MEDIAN (ng/mL)	ABSOLUTE RANGE (ng/mL)
7 - 8	35	2501	1082	3510	1250 - 6350
8 - 9	22	3539	665	3515	2300 - 5050
9 - 10	25	3694	794	3880	2190 - 5190
10 - 11	26	3382	1083	3160	1800 - 7060
11 - 12	26	3502	873	3305	2000 - 5470
12 - 13	27	3787	993	3840	1820 - 6990
13 - 14	27	4467	1119	4650	2400 - 7330
14 - 15	20	4652	1524	4885	1700 - 6940
15 - 16	19	4610	1494	4900	2100 - 7170
16 - 18	22	4625	1294	4315	2590 - 7280
18 - 20	22	4932	1218	5020	2680 - 7290
20 - 23	31	4645	1020	4490	2930 - 7380
23 - 25	22	4253	907	4415	2250 - 5480
25 - 30	52	3642	957	3405	2330 - 6680
30 - 40	43	3445	851	3350	1730 - 5590

Female

AGE (years)	N	MEAN (ng/mL)	SD (ng/mL)	MEDIAN (ng/mL)	ABSOLUTE RANGE (ng/mL)
7 - 8	21	4067	1137	4020	2060 - 6530
8 - 9	17	3923	830	4070	2560 - 5530
9 - 10	25	4440	1020	4350	2860 - 7740
10 - 11	24	4116	1115	4105	2690 - 7200
11 - 12	20	4771	1447	4200	2300 - 7740
12 - 13	23	4710	1699	4400	1800 - 8410
13 - 14	32	4310	1225	4170	2000 - 7110
14 - 15	24	4371	1089	4220	2600 - 7320
15 - 16	23	4440	944	4630	2400 - 5980
16 - 18	24	4319	1084	4190	2000 - 6470
18 - 20	21	4430	1530	4840	2310 - 7480
20 - 23	28	4668	1446	4340	2760 - 7350
23 - 25	20	4567	1319	4185	2920 - 7000
25 - 30	48	4220	1191	3985	2050 - 7600
30 - 40	29	3789	1114	3780	2300 - 7260

Both Sexes

AGE (years)	N	MEAN (ng/mL)	SD (ng/mL)	MEDIAN (ng/mL)	ABSOLUTE RANGE (ng/mL)
30 - 40	90	3493	900	3330	1730 - 7260
40 - 50	44	3152	497	3140	2080 - 4310
50 - 70	41	2966	439	2900	2020 - 3990

XIII. PERFORMANCE CHARACTERISTICS

A. Sensitivity:

The minimum detection limit, calculated as 2 standard deviations from the mean of 22 replicates of the 0 ng/mL IGFBP-3 Standard, is approximately 0.5 ng/mL.

B. Precision:

The intra-assay precision was determined for 3 serum samples containing low, mid-range, and high concentrations of IGFBP-3. Eight replicates of each sample were measured in the same assay, and the mean and SD values were obtained for each sample.

SAMPLE	N	MEAN (ng/mL)	STANDARD DEVIATION (ng/mL)	COEFFICIENT OF VARIATION (%)
I	8	82.72	1.53	1.8
II	8	27.53	0.90	3.2
III	8	7.35	0.29	3.9

The inter-assay precision was determined for 3 serum samples containing low, mid-range, and high concentrations of IGFBP-3. Replicates of each sample were measured in eight separate assays, and the mean and SD values were obtained for each sample.

SAMPLE	N	MEAN (ng/mL)	STANDARD DEVIATION (ng/mL)	COEFFICIENT OF VARIATION (%)
I	8	76.90	1.51	1.9
II	8	21.51	0.10	0.5
III	8	8.03	0.05	0.6

C. Recovery:

Pure IGFBP-3 was added in three different concentrations to stripped human serum containing a measured IGFBP-3 concentration of 11.0 ng/mL.

SAMPLE	ADDED (ng/mL)	EXPECTED (ng/mL)	OBSERVED (ng/mL)	RECOVERY (%)
I	5.0	16	16.06	100
II	50.0	61	64.37	106
III	100.0	111	95.90	86

D. Linearity of Dilution:

Three serum samples were diluted with the IGFBP-3 Sample Diluent and assayed.

SAMPLE	DILUTION FACTOR	EXPECTED (ng/mL)	OBSERVED (ng/mL)	RECOVERY (%)
I	—	—	75.95	—
	1:2	37.98	40.34	106
	1:4	18.99	22.05	116
	1:8	9.49	8.26	87
	1:16	4.74	4.29	89
	1:32	2.37	2.34	99
II	—	—	30.47	—
	1:2	15.23	15.10	99
	1:4	7.61	8.13	107
	1:8	3.81	4.32	113
	1:16	1.90	2.44	126
	1:32	0.95	1.15	116
III	—	—	8.58	—
	1:2	4.28	4.20	98
	1:4	2.14	2.47	115
	1:8	1.07	1.38	129
	1:16	0.54	0.54	100

E. Specificity:

1 µg/tube of the following peptide hormones gave concentrations of < 3 ng/mL:

PEPTIDE HORMONE
hIGFBP-1 (purified, amniotic fluid)
rhIGFBP-2
rhIGFBP-4
rhIGFBP-5
rhIGFBP-6
rhIGF-I
rhIGF-II

F. Interspecies Serum Comparisons:

NON-REACTIVE SERA	IMMUNOREACTIVE SERA
Sheep	Pig
Goat	Cow
Mouse	Rabbit
	Horse
	Dog
	Rat
	Guinea Pig

Since the actual concentrations of IGFBP-3 in the animal sera were not known and pure non-human IGFBP-3 was not available for testing, percent cross-reactivity could not be calculated. However, it is evident that none of the animal sera tested showed significant cross-reactivity or linearity in the DSL-6600 ACTIVE™ IGFBP-3 Coated-Tube IRMA.

G. Method Comparison:

The DSL-6700 IGFBP-3 RIA and the DSL-5600 IGF-I were compared to the DSL-6600 IGFBP-3 IRMA method by simultaneously assaying serum samples. The results are expressed below:

COMPARISON # 1: DSL-6600 IGFBP-3 IRMA vs. DSL-6700 IGFBP-3 RIA

n = 216
DSL-6600 Mean = 2986 ng/mL
DSL-6700 Mean = 3645 ng/mL
Sample Range = 700 - 9300 ng/mL
Slope = 0.66
Y-intercept = 553
Correlation Coefficient (r) = 0.84

COMPARISON # 2: DSL-6600 IGFBP-3 IRMA vs. DSL-5600 IGF-I IRMA

n = 383
DSL-6600 Mean = 3199 ng/mL
DSL-5600 Mean = 224 ng/mL
Sample Range = 150 - 8410 ng/mL
Slope = 3.9
Y-Intercept = 2307
Correlation Coefficient (r) = 0.62

XIV. REFERENCES

1. Daughaday W, Rotwein P: Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum and tissue concentrations. Endocr Rev 10:68-91, 1989.
2. Neely EK, Beaulieu MW, Oh Y, Cohen P, Rosenfeld RG.: Insulin-like growth factor receptors. Acta Paediatr Scand (suppl) 372:116-23, 1991.
3. Rosenfeld RG, Lamson G, Pham H, Oh Y, Conover C, De Leon DD, Donovan SM, Ocrant I, Giudice L.: Insulin-like growth factor-binding proteins. Rec Progr Horm Res 46:99-163, 1990.
4. Lamson G, Giudice L, Rosenfeld RG: Insulin-like growth factor binding proteins: structural and molecular relationships. Growth Factors. 5:19-28, 1991.
5. Cohen P, Fielder PJ, Hasegawa Y, Frisch H, Giudice LC, Rosenfeld RG: Clinical aspects of IGF binding proteins. Acta Endocrinol (Copenh.) 124 (suppl 2):74-85, 1991.
6. Baxter R, Martin J: Radioimmunoassay of growth hormone-dependent insulin like growth factor binding protein in human plasma. J Clin Invest 78:1504-1512, 1986.
7. Report on the nomenclature of the IGF binding proteins. Endocrinology 130: 1736-1737, 1992.
8. Wood WI, Cachianes G, Henzel WJ, Winslow GA, Spencer SA, Hellmiss R, Martin JL, Baxter RC: Cloning and expression of the GH dependent IGF binding protein. Mol Endocrinol 2:1176-1185, 1988.
9. Sprott SK, Tahara GP, Yamanaka MK, Ark BC, Delmar J, Maccarenhas D, Flynn J, Talkington-verser C, Spencer EM: Cloning and expression of human insulin-like growth factor binding protein 3. Growth Factors 3:65-72, 1990.
10. Hassenlopp P, Serrin D, Segovia-Quinson B, Hardouin S, Binoux M: Analysis of serum insulin-like growth factor binding proteins using Western blotting: use of the method for titration of the binding proteins and competitive binding studies. Anal Biochem 154:138-143, 1986.
11. Giudice LC, Farrell EM, Pham H, Lamson G, Rosenfeld RG: Insulin-like growth factor binding proteins in maternal serum throughout gestation and in the post-puerperium: effects of a pregnancy-associated serum protease activity. J Clin Endocrinol Metab 71:806-816, 1990.
12. Hassenlopp P, Segovia B, Lassace C, Raghani M, Bredon M, Binoux M: Evidence of enzymatic degradation of insulin-like growth factor binding proteins in the 150K complex during pregnancy. J Clin Endocrinol Metab 70:797-805, 1990.

IGFBP-3

Cohen P, Graves HCB, Peehl DM, Kamarei M, Giudice LC, Rosenfeld RG: Prostate specific antigen (PSA) is an IGF binding protein-3 protease found in seminal plasma. J Clin Endocrinol Metab 75: 1046-1053, 1992.

Gargosky SE, Hasegawa T, Hasegawa Y, Tapanen P, MacGillivray MH, Rosenfeld RG: Clinical potential of urinary insulin-like growth factor binding-protein-3 (IGFBP-3). 2nd International Workshop on Insulin-like Growth Factor Binding Proteins, Opio (Cote d'Azur), France, 1992. (abstract 93A)

Campbell PG, Novak JF, Yancsick TB, McMaster JH: Involvement of the plasmin system in dissociation of the insulin-like growth factor-binding protein complex. Endocrinology 130:1401-1412, 1992.

Blum WF, Ranke MB, Kietzman K, Gauggel E, Zeisel HJ, Bierich J: A specific radioimmunoassay for the growth hormone (GH)-dependent somatomedin binding protein: its use for diagnosis of GH deficiency. J Clin Endocrinol Metab 70: 1292-1298, 1990.

Rosenfeld RG, Wilson DM, Lee PDK, Hintz RL: Insulin-like growth factors I and II in evaluation of growth retardation. J Pediatr 109:428-33, 1986.

Powell DR, Rosenfeld RG, Baker BK, Liu F, Hintz RL:Serum somatomedin levels in adults with chronic renal failure: the importance of measuring insulin-like growth factor I (IGF-I) and IGF-II in acid chromatographed uremic serum. J Clin Endocrinol Metab 63:1186-1192, 1986.

Hasegawa Y, Hasegawa T, Aso T, Kotoh S, Tsuchiya Y, Nose O, Ohyama Y, Arai K, Tanaka T, Saiyo S, Yakaya S, Nishi Y, Miyamoto S, Sasaki N, Stene M: Usefulness and limitation of measurement of insulin-like growth factor binding protein-3 (IGFBP-3) for diagnosis of growth hormone deficiency. Endocrinol Japan 39(6): 585-591, 1992.

Guevara-Aguirre J, Rosenfeld R, et al.: Growth hormone receptor deficiency in Ecuador: clinical and biochemical phenotype in two populations. J Clin Endocrinol Metab 76: 417-423, 1993.

Miles LEM, Lipschitz DA, Bieber CP and Cook JD: Measurement of serum ferritin by a 2-site immunoradiometric assay. Analyt Biochem 61:209-224, 1974.