The Newcastle upon Tyne Hospitals NHS Trust
Department of Clinical Biochemistry
Freeman Hospital, Newcastle General Hospital
and
Royal Victoria Infirmary

Standard Operating Procedure for

SERUM INSULIN–LIKE GROWTH HORMONE (IGF-1)

SERUM INSULIN-LIKE GROWTH FACTOR (IGF-1)

PERSONNEL

All appropriately trained staff and trainees, under supervision.

PRINCIPLE

The Nichols Advantage IGF-1 Assay is a two site chemiluminescence immunoassay for the measurement of IGF-1 in human serum . The antibody to the C terminal with the amino acid sequence of 62-70 is biotinylated for capture and the antibody to the amino acid sequences of 1-23 and 42-61 is labelled with acridinium ester for detection. The patient's sample is acidified to separate IGF-1 from IGFBPs. Then excess IGF-2 is added in the assay to block the IGFBP binding sites fro recombining with the released IGF-1.
The acidified patient sample is incubated simultaneously with the biotinylated capture antibody, excess IGF-2, and the acridinium ester labelled tag antibody. During the first incubation, IGF-1 in the sample forms a sandwich complex with the capture antibody and the acridinium ester labelled antibody. After an initial incubation period streptavidin coated magnetic particles are added to the reaction mixture and a second incubation follows.
Free labelled antibody is separated from the labelled antibody bound to the magnetic particles by aspiration of the reaction mixture and subsequent washing. The wells containing the washed magnetic particles are transported into the system luminometer, which automatically injects the Triggers, initiating the chemiluminescence reaction. The light is quantitated by the luminometer and expressed as RLU. The amount of bound labelled antibody is directly proportional to the concentration of IGF-1 in the sample.

SAMPLE

A serum sample is required, no special precautions are needed. Once separated the samples are frozen and stored in Reception freezer prior to analysis.
On completion of the analysis, the samples are stored frozen for a minimum of 1 month.
The analysis is performed in singlicate, using 13ul of sample. Allowing for the dead volume, the minimum sample volume required is 213ul.
There are no specific requirements for patient preparation.
Grossly haemolysed, lipaemic and jaundiced samples can interfere with immunological reactions. Nichols recommend that such samples should not be run in the Advantage IGF-1 Assay. Refer to Nichols Advantage Assay Manual pages 14 & 15 for extensive results on interfering substances.

Do not use EDTA or heparinised samples since the IGF-1 values will be either lowered or highly variable.

SPECIFICITY

The two chromatographically purified antibodies show virtually no cross reactivity with other peptide hormones. The specificity of these antibodies was determined by spiking different hormones into samples and determining the change in response to unspiked samples. Refer to Nichols Advantage Assay Manual, page 11, for extensive results.

IMPRECISION

Within batch

	No.	x	SD	%CV
LEVEL I	20	23.99	1.90	7.90
LEVEL II	20	67.30	3.29	4.90
LEVEL III	20	83.65	4.77	5.70

Between batch

	No.	x	SD	%CV
LEVEL I	10	23.5	2.04	8.67
LEVEL II	10	71.57	3.58	5.00
LEVEL III	10	89.11	6.02	6.76

(Data source Nichols Advantage evaluation April 2000)

HEALTH AND SAFETY

Refer to the Departmental Safety Manual Index Code: **SAFETY1.DOC**

COSHH document **111** identifies the following compounds as hazards;

Assay Cartridges

Sodium Azide	Toxic by ingestion Irritating to eyes and skin.
Proclin 300	Harmful by ingestion. Irritating to skin and eyes.
EDTA Sodium salt	Irritating to skin and eyes. May be harmful if ingested in quantity.

IGF –1 sample Acidifier

HCl	Irritating to eyes and skin, and may cause burns if contact is prolonged. Ingestion may cause internal irritation.

System Trigger Set

Hydrogen Peroxide 30%	Causes severe burns to skin and eyes. Extremely irritating to respiratory system. If ingested sudden evolution of oxygen may cause injury by acute distension of the stomach and may cause nausea, vomiting and internal bleeding.
Nitric Acid (Conc)	Causes severe burns to eyes and skin. If ingested causes severe internal irritation and damage. Irritating harmful vapour.
Sodium Hydroxide 50%	Corrosive to body tissue causing burns and deep ulceration. If ingested causes severe internal irritation and damage. Emits toxic fumes under fire conditions.

ALL REAGENTS SHOULD BE CONSIDERED HARMFUL BY INGESTION

RISK ASSESSMENT

The instrument, sample and waste handling risks are covered by the Nichols Advantage Risk Assessment in the instrument SOP Index Code ; ADVAN.DOC.

PREPARATION OF CALIBRATORS & REAGENTS

IGF-1 calibrators A & B (Cat No.63-7207) are provided by the manufacturer. They are stored in lyophilised form at 4ºC until use when they are reconstituted with 1 ml of osmotically purified water. After 20 minutes at room temperature they can be aliquoted into 3x 0.33ml amounts and stored frozen (-40ºC). Bar codes are provided with the calibrators for instrument identification.

IGF-1 reagent cartridges (Cat. No.62-7007) sufficient for 50 tests, are provided by the manufacturer and include all reagents required for analysis. These are stored at 4ºc in the cold room in M1242. Prior to opening a new cartridge it should be inverted 3 times before removing the gold seals. The cartridge is placed on the M-Prep Accelerator, situated next to the analyser, for 2-3 minutes to lift the magnetic particles. Once lifted the particles should be agitated vigorously before inserting into the reagent drawer on the analyser. Check there are no bubbles on the surface of the liquids.

QUALITY CONTROL

Internal QC and external QA must be performed as defined for this chemistry in QC1.DOC.

EQUIPMENT USED

Refer to Nichols Advantage operating procedure Index Code; ADVAN.DOC.

CALIBRATION

Calibration is required once a week and is prompted by the analyser, or with the introduction of a new batch of cartridges or as indicated by internal QC performance. Recalibration is also recommended after a visit by the service engineer.

IGF-1 is referenced against WHO 1st International Reference Reagent 1988, Insulin Like Growth Factor 1 87/518.

PROCEDURE

Refer to Nichols Advantage operating procedure Index Code; ADVAN.DOC.

CALCULATION AND VERIFICATION OF DATA

The Nichols Advantage automatically calculates the results from the current calibration curve and reports these results in the appropriate units. Any error flags appearing beside the results need to be investigated and the appropriate action taken (refer to user manual for extensive list of error codes).
Refer to the QC document Index Code: QC1.DOC for the current QC ranges and acceptability.

REPORTING OF RESULTS

Results are reported to the nearest whole number in nmol/l.
The sensitivity of the Nichols IGF-1 assay has been calculated to be 0.8 nmol/l.
The lower limit of reporting is less than 2 nmol/l and is reported as <2.
There is no upper limit of reporting as a sample giving a result above that of the highest standard would be diluted appropriately in sample diluent (Cat.No.64-7707) to give an absolute value.

Results are transferred from the Advantage printout to the Apex work document, then the results are entered manually into Apex at authorisation level F (refer to Computer document Index Code; APEX.DOC for the generation of work documents and for the entry of results).

REFERENCE RANGE

AGE(YEARS)	MALE	FEMALE
Less than 1	4-14	2-9
1-2	4-14	2-9
3-4	8-23	5-29
5-6	11-15	7-43
7-8	11-33	13-35
9-10	16-35	24-46
11-12	25-37	24-78
13-14	21-87	37-103
15-16	32-84	48-91
17-18	20-56	35-73
19-20	21-85	21-51
21-25	18-42	12-44
26-39	15-37	12-44
40-54	14-32	12-44
55-88	11-30	12-44

LIMITATIONS

Efforts by Nichols have been made to minimise heterophilic antibody interference, however specimens from patients who have received mouse monoclonal antibodies may give falsely elevated or suppressed results.

CLINICAL SIGNIFICANCE

IGF-1 is a 70 amino acid straight chain peptide of M.Wt. 7649 Daltons. It is part of a family of growth factors which include IGF-1, 2, and Somatomedins A and B and MSA. IGF-1 is highly basic in nature and circulates bound to high M.wt. binding proteins of approximately 140,000 Daltons.

It mediates the growth promoting action of GH and is released from a variety of body tissues in response to GH and is under a feedback control mediated via the hypothalamic/pituitary loop. Other than GH, the most important factors influencing IGF-1 concentration are age and sex. Peak levels are seen during adolescence, lowest levels at birth.

Measurement of IGF-1 is advocated along with GH measurement to:-

1. Investigate GH deficiency in children.

2. Diagnosis and treatment of Acromegaly (especially of Bromocriptine treated cases).

REFERENCES

1. Blum WF, Breier BH (1994). Radioimmunoassays for IGFs and IGFBPs, Third International Symposium On IG Factors, Sydney Australia July, pp11-19.
2. Nichols Institute Diagnostics Directional Insert fir IGF-1 Extraction April 1997.
3. Nichols Advantage User Manual and Assay Manual.