



# INSTRUCTION MANUAL

IVD

( July 21<sup>st</sup>, 2005 )

## CentAK<sup>®</sup> anti-IA<sub>2</sub>

- 50 determinations -

REF 1750



Radioligand assay for the determination of autoantibodies to Protein Tyrosine Phosphatase IA<sub>2</sub> in human serum



**MEDIPAN GMBH**

Ludwig-Erhard-Ring 3

**15827 Dahlewitz / Berlin (Germany)**

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Phone: +49(0)33 708 / 44 17 - 0

Fax: +49(0)33 708 / 44 17 - 25

info@medipan.de  
www.medipan.com

### INTENDED USE

Type 1 diabetes, also known as insulin-dependent diabetes mellitus (IDDM), results from a chronic autoimmune destruction of the insulin-secreting pancreatic beta cells, probably initiated by exposure of genetically susceptible host to an environmental agent. Autoimmune destruction of beta cells is thought to be completely asymptomatic until 80 - 90 % of the cells are lost. This process may take years to complete and may occur at any time.

During the preclinical phase, this autoimmune process is marked by circulating autoantibodies to beta cell antigens. These autoantibodies are present years before the onset of type 1 diabetes and prior to clinical symptoms. Early studies utilized the immunofluorescence test for islet-cell antibodies (ICA), which has been difficult to standardize and is now replaced by a combination of several radioimmunoassays for antibodies against specific beta cell antigens, such as insulin (IAA), glutamic acid decarboxylase (GAD) and tyrosine phosphatase ICA 512 (IA<sub>2</sub>).

IA<sub>2</sub>, a member of the protein tyrosine phosphatases family is localized in the dense granules of pancreatic beta cells and the second defined recombinant islet cell antigen. IA<sub>2</sub> shares sequence identity with the islet cell antigen 512. The higher frequency of antibodies to IA<sub>2</sub> is explained by the presence of autoantibodies directed to the COOH terminus of IA<sub>2</sub> which is lacking in the ICA512 molecule.

IA<sub>2</sub> autoantibodies are present in the majority of individuals with new-onset type 1 diabetes and in individuals in the pre-diabetic phase of the disease. The appearance of autoantibodies to IA<sub>2</sub> seems to be correlated with the rapid progression to overt type 1 diabetes.

The combination of tests for GAD<sub>65</sub> and IA<sub>2</sub> autoantibodies is highly relevant for risk assessment of type 1 diabetes in children and adolescence. The screening for GAD<sub>65</sub> and IA<sub>2</sub> autoantibodies detect more than 90 % of subjects at risk for type 1 diabetes and may, therefore, possess the potential to replace ICA technique.

### PRINCIPLE of the TEST

CentAK<sup>®</sup> anti-IA<sub>2</sub> is a direct assay based on the principle of radioligand assays. Highly purified human recombinant IA<sub>2</sub> (intracellular fragment of IA<sub>2</sub>) is labeled with 125-Iodine. This tracer is used in excess and bound by the IA<sub>2</sub> autoantibodies of the sample.

CentAK<sup>®</sup> anti-IA<sub>2</sub> tracer meets the highest requirements with regard to purity, enzymologic identity, fast reaction kinetics, cross reactivity at zero level and stability. These are the main prerequisites for the specific binding of the tracer and its exclusive recognition by the IA<sub>2</sub> autoantibodies of the sample.

By adding Protein A (staphyl. aureus) which binds to the Fc moiety of the autoantibodies, sandwich-type complexes are formed. This solid phase facilitates the simple separation of the bound fraction (B) by centrifugation. After removing the supernatant which contains the non-bound tracer by aspiration or decantation, the radioactivity of the remaining precipitate is measured.

The concentration of IA<sub>2</sub> autoantibodies (anti-IA<sub>2</sub>) in the sample is reflected by the specifically bound tracer amount. The radioactive signal (cpm) of the bound fraction (B) is proportional to the autoantibody concentration.

No immune complex is formed if autoantibodies against IA<sub>2</sub> are absent in the sample, as the tracer binds solely to IA<sub>2</sub> autoantibodies, but not to Protein A.

**A standard curve with a range of 0.1 - 50 U/ml** is established by measuring cpm respectively the binding B/T % of the calibrators **1 - 5**. The anti-IA<sub>2</sub> concentration value of the patient's sample is directly read off against this curve.

#### IFU symbols radioactive assays MEDIPAN GMBH



In vitro diagnostic device



EC Declaration of Conformity



Catalogue number



Batch code



Expiry date



Manufactured by



Consult accompanying documents



Consult operating instruction



Store at



Biological risk



Radioactive component



Tracer



Buffer



Control serum



Calibrators



Protein A-suspension

## PATIENT SAMPLES


### Specimen collection and storage

Blood is taken by venipuncture. After clotting, the serum is separated by centrifugation. Plasma is also suitable for use in CentAK<sup>®</sup> anti-IA<sub>2</sub>. Lipaemic and hemolytic samples should not be employed.

The samples may be kept at 2 - 8 °C up to three days. Long-term storage requires - 20 °C.

Repeated freezing and thawing should be avoided. For multiple use, initially aliquot samples and keep at - 20 °C.

## TEST COMPONENTS for 50 DETERMINATIONS

<b>D</b> <b>TRAC</b>	<b>Tracer (125-I- A<sub>2</sub> human, recombinant)</b> < 0.05 MBq		1 vial, lyophilized, reconstitution: 2.6 ml J
<b>J</b> <b>BUF D</b>	<b>Buffer</b> (for reconst. of components D and L and or washing)		1 vial, 120 ml, ready for use
<b>L</b> <b>PRE</b>	<b>Protein A-suspension</b>		1 vials, lyophilized, reconst.: 2.6 ml J
<b>1 - 5</b> <b>CAL</b>	<b>Anti-IA<sub>2</sub>-Calibrators</b> (human serum) conc.: 0.1; 0.75; 2.0; 10; 50 U/ml		5 vials; 0.15 ml, each, ready for use
<b>CI - CII</b> <b>CONTROL</b>	<b>Anti- IA<sub>2</sub>-Control sera</b> (human sera) conc.: cf. leaflet enclosed		2 vials; 0.15 ml, each, ready for use

### Size and storage

CentAK<sup>®</sup> anti-IA<sub>2</sub> has been designed for 50 determinations. This is sufficient for the analysis of 18 unknown samples as well as for calibrators and control sera, assayed in duplicates.

The expiry date of each component is reported on its respective label, that of the complete kit on the box label.

Upon receipt, all components of the CentAK<sup>®</sup> anti-IA<sub>2</sub> have to be kept at 2 - 8 °C, preferably in the original kit box.

### Preparation before use

Allow all of the components to reach room temperature prior to use in the assay.

- D Tracer:**  
Reconstitute with 2.6 ml J per vial. Reconstituted tracer remains stable for 2 weeks, stored at 2 - 8 °C.
- J Buffer:**  
BUFD is ready for use and serves for the reconstitution of the tracer and the Protein A-suspension as well as for washing.
- L Protein A-Suspension:**  
Reconstitute with 2.6 ml J per vial. The reconstituted suspension remains stable for 2 weeks stored at 2 - 8 °C.

**Note:** Protein A suspension tends to precipitate in rest, thus agitate bottle end over end gently for 10 - 20 seconds **immediately before use**. This is not necessary for the short time of taking aliquots for the assay procedure.

**1 - 5 Calibrators:** Ready for use.

**CI - CII Control sera:** Ready for use.

## ASSAY PROCEDURE

Use conical tubes for CentAK<sup>®</sup> anti-IA<sub>2</sub> only.

1. Label test tubes appropriately.
2. Pipette into the corresponding tubes according to assay scheme
  - 20 µl calibrators,
  - 20 µl control sera,
  - 20 µl patient's samples, each.
3. Add 50 µl tracer (prepared from D and J), each, to **all tubes**, including those for total radioactivity T.  
*Tubes T are now separated until radioactivity is measured.*
4. Incubate over night (at least 18 hours at 4 - 8 °C).
5. Add 50 µl Protein A-suspension (prepared from J and L), each.  
(Agitate the suspension gently prior to use - please section Test Components, preparation before use).
6. Incubate 60 minutes (at room temperature).
7. Add 1 ml buffer (J), each.
8. Centrifuge the tubes for 20 minutes at a minimum of 1500 x g.
9. Aspirate supernatant completely or decant. For removal of any remaining liquid, turn tubes upside down (5 - 10 minutes) and absorb any droplets by tapping on blotting paper.
10. Measure radioactivity of **all tubes including T**.  
Recommended counting time: 1 minute

## DATA PROCESSING

### We recommend log/log processing for best results!

The standard curve is established by plotting the mean cpm-values of the calibrators 1 - 5 on the ordinate, y-axis, (log. scale) versus their respective anti-IA<sub>2</sub>-concentrations on the abscissa, x-axis, (log. scale).

The anti-IA<sub>2</sub> concentrations of the controls and the unknown samples are **directly read off** in U/ml against the respective cpm values.

The respective binding rates B, related to the total radioactivity T may be used as well for setting up the standard curve (B/T %).

CentAK<sup>®</sup> anti-IA<sub>2</sub> may be used also with Computer Assisted Analysis using software able to plot log/log curves with spline smoothing, such as for sandwich-type assays (IRMA).

### We recommend log/log processing for best results!

## TYPICAL EXAMPLE

(approx. 4 weeks before expiry)

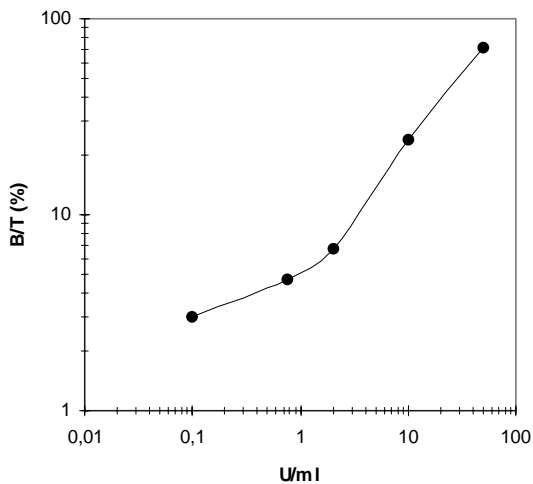
**Do not use for evaluation!**

Test tubes	cpm (a)	cpm (b)	cpm (mean)	$\frac{B}{T}$ %	U/ml
Total radioactivity T	33533	34136	33833	100 %	---
Calibrator 1	1078	920	999	3.0	<b>0.10</b>
Calibrator 2	1616	1532	1574	4.7	<b>0.75</b>
Calibrator 3	2334	2207	2270	6.7	<b>2.00</b>
Calibrator 4	8323	7882	8102	23.9	<b>10.00</b>
Calibrator 5	24341	23612	23976	70.9	<b>50.00</b>
Control I	---	---	---	---	---
Control II	---	---	---	---	---
Patient 1	4330	4322	4326	12.8	<b>4.8</b>

Calculation of patient sample 1:  $\frac{B}{T} (\%) = \frac{4326}{33833} \times 100 = 12.8 \%$

## STANDARD CURVE

Typical example



## REFERENCE VALUES

CentAK® anti-IA <sub>2</sub>	
IA <sub>2</sub> antibodies negative	< 0.75 U/ml
IA <sub>2</sub> antibodies positive	≥ 0.75 U/ml

It is recommended that each laboratory establishes its own normal and pathological reference ranges as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Normal range was established by evaluation of data from patients with type 1 diabetes and healthy control subjects.

## CHARACTERISTIC ASSAY DATA

### Calibration

The units in the CentAK® anti-IA<sub>2</sub> are arbitrary units.

CentAK® anti-IA<sub>2</sub> assay based on <sup>125</sup>I-IA<sub>2</sub> shows good agreement with the <sup>35</sup>S labeled IA<sub>2</sub> precipitation assay (r = 0.88; n = 141).

CentAK® anti-IA<sub>2</sub> passed the 3rd IA<sub>2</sub>Ab Proficiency Study of the University of Louisiana, New Orleans, USA, in 1999, with 100 % specificity and 100 % sensitivity.

### Parallelism of standards and serum samples

Dilutions of specimen in anti-IA<sub>2</sub> free human serum are determined according to their expected theoretical values with CentAK® anti-IA<sub>2</sub>.

On the basis of the heterogeneous nature of the autoantibody population in human serum and in view of epitope specificity and affinity of the autoantibodies in some cases are not determined the expected theoretical values.

### Specificity

The high quality of the tracer (<sup>125</sup>I-IA<sub>2</sub>) does secure in direct assay principle of the test, that only anti-IA<sub>2</sub> autoantibodies react and that any detectable cross reactions with autoantibodies to GAD<sub>65</sub>, Thyroglobulin, thyroidal Peroxidase, to the TSH receptor and Acetylcholine receptor do not exist.

### Sensitivity (lower detection limit)

The most appropriate and statistically reasonable definition of the lower detection limit of any assay is at present the so-called **functional assay sensitivity**.

This functional assay sensitivity generally represents that concentration which corresponds to the 10 % (within-assay) and to the 20 % (between assay) coefficient of variation in the respective precision profiles of the assay in the lower concentration range. Upon correct and thorough performance of CentAK® anti-IA<sub>2</sub>, this value is found at approx. 0.7 U/ml.

Anti-IA<sub>2</sub> values below this defined level of functional assay sensitivity do not meet the statistical criteria for reliability according to GLP (Good Laboratory Practice) and therefore can not be distinguished from zero due to the statistically necessary certainty.

Anti-IA<sub>2</sub> concentrations above approx. 0.7 U/ml, however, fulfill these criteria and are consequently assessed as valid.

## LIMITATIONS of the METHOD

Healthy individuals should be tested negative by using the CentAK® anti-IA<sub>2</sub>.

However, IA<sub>2</sub> autoantibodies may be also present in a rare neurological disorder, Stiff-man Syndrome (SMS). In sera from patients with SMS IA<sub>2</sub> autoantibodies appear seldom.

Any clinical diagnosis should not be based on the results of in vitro diagnostic method alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

# CentAK<sup>®</sup> anti-IA<sub>2</sub>

## ASSAY SCHEME

1	Label test tubes*	CAL 1 - 5	CI - CII	Pat.-Sera 1, 2 etc.	T
2	Pipette Calibrators 1 - 5 Control sera I - II Patient sera	20 µl	20 µl	20 µl	
3	Pipette Tracer (prepared from D and J)	50 µl	50 µl	50 µl	50 µl
4	Incubate**	over night (at least 18 hours) (at 4 - 8 °C)			
5	Pipette Protein A-Suspension (prepared from J and L)	50 µl	50 µl	50 µl	
6	Incubate**	60 minutes (at room temperature)			
7	Pipette BUFD (J)	1 ml	1 ml	1 ml	
8	Centrifuge	20 minutes at 1500 x g			
9	Decant supernatant or Aspirate supernatant	leave tubes upside down on absorbent paper for 5 to 10 minutes quantitatively			
10	Count radioactivity	Counting time: 1 minute			

\* use conical tubes

\*\* Prior to incubation, agitate the tubes briefly in order to ensure homogeneous reaction conditions.

## SAFETY PRECAUTIONS

- **This kit is for in vitro use only.** Follow the working instructions carefully.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts (< 0.1 % w/w) of sodium acid as a preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBSAg and for HIV as well as HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- It is the responsibility of the user of this product to handle radioactive material in accordance to the national rules given by law or other statements of the local authorities.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP with all general and individual regulations has to be applied to the use of this kit.

