



INSTRUCTION MANUAL

IVD

(July 28th, 2010)

CentAK[®] IAA RT

- 100 determinations -

REF 1735



Radioligand assay for the determination of **Autoantibodies to Insulin (IAA)** in human serum



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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



Anti-human-IgG



Tracer



Buffer



Control serum



Calibrators

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 in all ages.

The presence of **Insulin autoantibodies (IAA)** in patients **never treated with insulin**, as opposed to insulin antibodies (IAb), is evidence of ongoing destruction process of pancreatic beta cells in type 1 diabetes. **IAA** are particularly important when determining type 1 diabetes risk since their prevalence is significantly elevated in subjects developing the disease in childhood and moreover, they are often the first autoantibodies to be detected before onset of the disease. The prevalence of IAA is inversely correlated with the age of diagnosis.

In type 1 diabetics with recent onset of the disease in the age < 5 years IAA can be determined in > 90 % of the patients, whereas in type 1 diabetics in the age > 20 years the prevalence of IAA is < 20 %.

As concluded by the Fourth International Workshop for Insulin Autoantibody (IAA) Standardization (1992), liquid-phase assays such as radio-binding assay - RIA is the method of choice - detect IAA of higher predictive value for type 1 diabetes as ELISA.

The IAA measurement, together with that of antibodies to glutamic acid decarboxylase (GAD65 Ab), protein tyrosine phosphatase-like antigen IA2 forms the basis of current strategies for predicting future onset of type 1 diabetes.

LITERATURE

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PRINCIPLE of the TEST

CentAK[®] IAA RT is a direct assay based on the principle of radioligand assays for the measurement of IgG-specific IAA.

Highly purified human 125-I-(A14) mono-iodinated insulin is used in the CentAK[®] IAA RT. This tracer meets the highest requirements with regard to purity, 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 IAA of the sample in the first incubation step. This is followed in a second incubation step by addition of anti-human-IgG to precipitate any labeled insulin-anti insulin complexes which have formed. 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 Insulin autoantibodies (IAA) 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 IAA are absent in the sample. The IAA value of the patient's sample is directly read off against this curve.

PATIENT SAMPLES




Specimen collection and storage

Blood is taken by venipuncture. After clotting, the serum is separated by centrifugation. Plasma, 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 100 DETERMINATIONS

D	Tracer (125-I-Insulin, human, recombinant) < 0.04 MBq each per vial $t_{1/2} = 59$ days gamma radiation 35 keV, x radiation 27 keV, 31 keV		2 vials, lyophilized, red colored reconstitution: 1.5 ml J, each
TRAC			
J	Buffer (for reconstitution of components D and for washing steps)		2 bottles, 250 ml each, ready for use
BUF D			
L	Anti-human-IgG		2 vials, 5.5 ml, each, ready for use
PRE			
0	Negative control serum		1 vial, 0.5 ml, ready for use
CAL			
1 - 4	Calibrators (human serum) conc.: cf. leaflet enclosed		4 vials; lyophilized, reconstitution: 0.25 ml dist. water, each
CAL			
CI - CII	Control sera (human sera) conc.: cf. leaflet enclosed		2 vials; lyophilized, reconst.: 0.25 ml dist. water, each
CONTROL			

Materials required

- Precision pipettes 20 - 100 µl, 1000 µl
- Disposable pipette tips
- Distilled or de-ionized water
- Absorbent paper or paper towel
- Vortexer
- γ -counter

Size and storage

CentAK[®] IAA RT has been designed for 100 determinations. This is sufficient for the analysis of 41 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[®] IAA RT have to be kept at 2 - 8 °C, preferably in the original kit box.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

- D Tracer:**
Reconstitute with 1.5 ml J per vial. Reconstituted tracer remains stable for 4 weeks, stored at 2 - 8 °C, for long-term storage, - 20 °C are necessary.
- J Buffer:**
BUFD is ready for use and serves for the reconstitution of the tracer as well as for washing steps.
- L anti-human-IgG:**
5.5 ml each, containing precipitation enhancer, ready for use.

- 0 Negative control serum:**
0.5 ml, ready for use. Negative control serum can be used also as so-called **zero-calibrator**.
Negative control serum should be assigned a value of 0.05 U/ml to assist in computer processing of assay results.

- 1 - 4 Calibrators:**
Reconstitution: each vial with 0.25 ml distilled water.
Concentration: cf. leaflet enclosed.

- CI - CII Control sera:**
Reconstitution: each vial with 0.25 ml distilled water.
Concentration: cf. leaflet enclosed

The reconstituted standards and controls may be kept at 2 - 8 °C for up to 2 months.

ASSAY PROCEDURE

Please, use round bottom tubes.

1. Label test tubes appropriately.
2. Pipette into the corresponding tubes according to assay scheme
 - 20 µl calibrators,
 - 20 µl negative control serum,
 - 20 µl control sera I + II,
 - 20 µl patient's samples 1, 2, ...
3. Pipette 25 µl tracer (prepared from D and J), 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 room temperature (RT 20 - 25 °C).
5. Pipette 100 µl anti-human IgG (L), each.
(Agitate the suspension gently prior to use - please cf. section Test Components, preparation before use).
6. Incubate for **1 hour** at 4 - 8 °C.
7. Pipette in each tube
2 ml of cold (2 - 8 °C) buffer J
8. Mix the tubes by vortexing and centrifuge the tubes for 20 minutes at a minimum of 1500 x g at 4 °C.
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. Pipette in each tube 2 ml J.
11. Repeat step 8 + 9.
12. Measure radioactivity of **all tubes including T**. Recommended counting time: 1 minute

DATA PROCESSING

The calibrator curve is established by plotting the mean cpm-values of the calibrators 1 - 4 on the ordinate, y-axis, (lin. scale) versus their respective IAA-concentrations on the abscissa, x-axis, (log. scale).
The IAA-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[®] IAA RT may be used also with Computer Assisted Analysis using software able to plot lin/log curves with spline smoothing, such as for sandwich-type assays (IRMA).

We recommend lin / log processing for best results!

TYPICAL EXAMPLE

(approx. 4 weeks before expiry)

Do not use for evaluation!

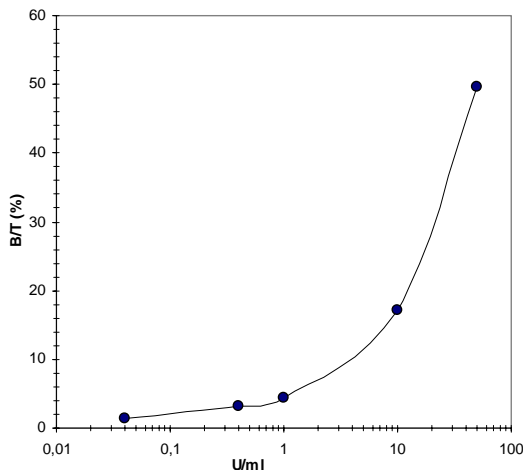
Test tubes	cpm (a)	cpm (b)	cpm (mean)	$\frac{B}{T}$ %	U/ml
Total radioactivity T	28153	29138	28646	100 %	---
Neg. Control	392	403	398	1.4	0.04
Calibrator 1	901	945	923	3.2	0.4
Calibrator 2	1199	1256	1228	4.3	1
Calibrator 3	5001	4799	4900	17.1	10
Calibrator 4	13997	14463	14230	49.7	50
Control I	---	---	---	---	---
Control II	---	---	---	---	---
Patient 1	5990	5777	5884	20.6	13.3

Calculation of patient sample 1:

$$\frac{B}{T} (\%) = \frac{5884}{28646} \times 100 = 20.6 \%$$

STANDARD CURVE

Typical example



REFERENCE VALUES

CentAK® IAA RT	
IAA negative	< 0.4 U/ml
IAA positive	≥ 0.4 U/ml

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

CHARACTERISTIC ASSAY DATA

Calibration

The units in the CentAK® IAA RT are arbitrary units.

Parallelism of standards and serum samples

Dilutions of specimen in IAA free human serum are determined according to their expected theoretical values with CentAK® IAA RT. 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 (125-I-A14-monoiodinated Insulin) does secure in direct assay principle of the test, that only IAA react and that any detectable cross reactions with autoantibodies to IA2, GAD65, Thyroglobulin, thyroidal Peroxidase, to the TSH receptor and Acetylcholine receptor do not exist.

Sensitivity (lower detection limit)

Analytical assay sensitivity is measured as 10 fold repetition of the negative control serum + 3 SD. It is determined to be 0.1 U/ml.

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 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® IAA RT this value is found at approx. 0.2 U/ml.

IAA 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.

IAA concentrations above approx. 0.2 U/ml, however, fulfill these criteria and are consequently assessed as valid.

Intra-assay			Inter-assay		
Sample no	Mean concentration (U/ml)	CV (%)	Sample no	Mean concentration (U/ml)	CV (%)
1	0.06	14	6	0.1	35
2	0.2	8	7	0.5	11
3	3.7	4	8	1.6	13
4	5.8	6	9	8.9	11
5	32.1	3	10	28.8	9

LIMITATIONS of the METHOD

Healthy individuals should be tested negative by using the CentAK® IAA RT.

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[®] IAA RT

ASSAY SCHEME

1	Label test tubes	CAL 0	CAL 1 - 4	CI - CII	Pat. sera 1, 2 etc.	T
2	Pipette negative control (CAL 0) Calibrators 1 - 4 Control sera I + II Patient's sera 1, 2 etc.	20 µl	20 µl	20 µl	20 µl	
3	Pipette Tracer (prepared from D and J)	25 µl	25 µl	25 µl	25 µl	25 µl
4	Incubate*	over night (at least 18 hours) at room temperature (RT 20 - 25 °C)				
5	Pipette anti-human-IgG (L)	100 µl	100 µl	100 µl	100 µl	
6	Incubate*	1 hour 4 - 8 °C				
7	Pipette BUFD (J) cold (2 - 8 °C)	2 ml	2 ml	2 ml	2 ml	
8	Centrifuge	Mix the tubes by vortexing and centrifuge at 1500 x g for 20 minutes at 4 - 8 °C				
9	Decant supernatant or Aspirate supernatant	leave tubes upside down on absorbent paper for 5 - 10 minutes or quantitatively				
10	Pipette BUFD (J) cold (2 - 8 °C)	2 ml	2 ml	2 ml	2 ml	
11	Repeat step 8 and 9					
12	Count radioactivity	Counting time: 1 minute				

* 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. This instruction manual is valid only for the present kit with the given composition. An exchange of single components is not in agreement with CE regulations.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted 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 %) of sodium azide as a preservative. They must not be swallowed or allowed to come into contact with skin or mucosa. The possible formation of heavy metal azides in the drainage has to be prevented by sufficient rinsing with water.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for both Hepatitis and HIV antibody. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains radioactive material the following precautions should be observed:
 - Do not smoke, eat or drink while handling radioactive material in any room designated for working with radioactive material,
 - Always use protective gloves,
 - Never pipette radioactive material by mouth,
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant,
 - Place contaminated tissues, tubes, bench covers, gloves etc. in a specially marked container, discard liquid and solid radioactive waste only as permitted by federal, state or local authorities and regulations.
- 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.
- In any case GLP with all general and individual regulation has to be applied to the use of this kit.

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