



INSTRUCTION MANUAL

IVD

(July 21st, 2005)

CentAK[®] anti-GAD₆₅

- 100 determinations - REF 1700 -

- 50 determinations - REF 1720 -



Radioligand assay for the determination of autoantibodies to **Glutamic Acid Decarboxylase (GAD₆₅Ab)** in serum



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IFU symbols radioactive assays MEDIPAN GMBH			
IVD	In vitro diagnostic device	CE	EC Declaration of Conformity
REF	Catalogue number	LOT	Batch code
	Expiry date		Manufactured by
	Consult accompanying documents		Consult operating instruction
	Store at		Biological risk
	Radioactive component		
TRAC	Tracer	BUF D	Buffer
CONTROL	Control serum	CAL	Calibrators
PRE	Protein A-suspension		

Manufactured under license to patents including US 5,512,447 and EP 0502188

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.

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 (IA₂).

GAD, the enzyme that catalyzes the conversion of glutamate to GABA, has been identified in two isoforms, molecular weight 65.000 (GAD₆₅) and 67.000 (GAD₆₇). Although GAD autoantibodies are found in type 1 diabetes and in the rare neurological disorder Stiff man syndrome (SMS), the GAD autoantibodies profile in the two diseases differs.

Autoantibodies of SMS patients recognize a combination of linear and conformational epitopes of GAD while GAD autoantibodies in patients with type 1 diabetes are predominantly directed to the conformational epitopes. **GAD₆₅ autoantibodies are present in 70-80 % of newly diagnosed patients with type 1 diabetes** and can be detected many years before clinical onset of the disease

The combination of the autoantibodies to GAD₆₅ and IA₂ is highly relevant for risk assessment of type 1 diabetes in children and adolescence. These tests in combination are more sensitive and predictive than ICA in risk groups, e.g. relatives of type 1 diabetes.

GAD₆₅ autoantibodies also occur in a subset of adults with type 2 diabetes (non-insulin-dependent diabetes mellitus). These patients can have pronounced hyperglycemia, and after therapy with oral hypoglycemic agents for several months to years they may become insulin dependent. Therefore, these patients are thought to have a slowly progressive form of type 1 diabetes, sometimes called latent diabetes or **latent autoimmune diabetes in adults (LADA)**.

The presence of GAD₆₅ autoantibodies in sera of such patients is a sensitive and specific marker for future insulin dependency.

PRINCIPLE of the TEST

CentAK[®] anti-GAD₆₅ is a direct assay based on the principle of radioligand assays. Highly purified human recombinant GAD₆₅ is labeled with I¹²⁵-Iodine. This tracer is used in excess and bound by the GAD₆₅ autoantibodies of the sample.

CentAK[®] anti-GAD₆₅ 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 GAD₆₅ 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 GAD₆₅ autoantibodies (anti-GAD₆₅) 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 GAD₆₅ are absent in the sample, as the tracer binds solely to GAD₆₅ autoantibodies, but not to Protein A.

A standard curve with a range of 0.1 - 120 (300) U/ml is established by measuring cpm respectively the binding B/T % of the calibrators **1 - 6 (7)**. The anti-GAD₆₅ concentration 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 is also suitable for use in CentAK® anti-GAD₆₅. 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 (50) DETERMINATIONS

D TRAC	Tracer (125-I-GAD₆₅, human, recombinant) < 0.05 MBq per vial	 2 (1) vials, lyophilized, reconstitution: 2.6 ml J, each
J BUFD	Buffer (for reconstitution of components D and L and for washing)	1 bottle, 120 ml, ready for use
L PRE	Protein A-suspension	2 (1) vials, lyophilized, reconst.: 2.6 ml J, each
1 - 7 CAL	Anti-GAD₆₅-Calibrators (human serum) conc.: 0.1; 1.0; 3.0; 10; 30; 120 U/ml (300 U/ml optional)	7 vials; 0.15 ml, each, ready for use
CI - CII CONTROL	Anti-GAD₆₅-Control sera (human sera) conc.: cf. leaflet enclosed	2 vials; 0.15 ml, each ready for use

Size and storage

CentAK® anti-GAD₆₅ has been designed for 100 and 50 determinations, respectively. This is sufficient for the analysis of 41 or 16 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® anti-GAD₆₅ 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 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 - 6 (7) Calibrators: Ready for use.

Normally, the calibrators 1 - 6 (0.1 - 120 U/ml) are used for the preparation of the standard curve. Expecting high values of antibody titers the optional calibrator 7 (300 U/ml) can be used for larger range of the standard curve.

CI - CII Control sera: Ready for use.

ASSAY PROCEDURE

Use conical tubes for CentAK® anti-GAD₆₅ 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 for 2 hours (at room temperature).
5. Add 50 µl Protein A-suspension (prepared from J and L), each.

(Agitate the suspension gently prior to use - please cf. section Test Components, preparation before use).
6. Incubate for 1 hour (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

The standard curve is established by plotting the mean cpm-values of the calibrators 1 - 6 (7) on the ordinate, y-axis, (log. scale) versus their respective anti-GAD₆₅-concentrations on the abscissa, x-axis, (log. scale, as well).

The anti-GAD₆₅ 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® anti-GAD₆₅ 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)

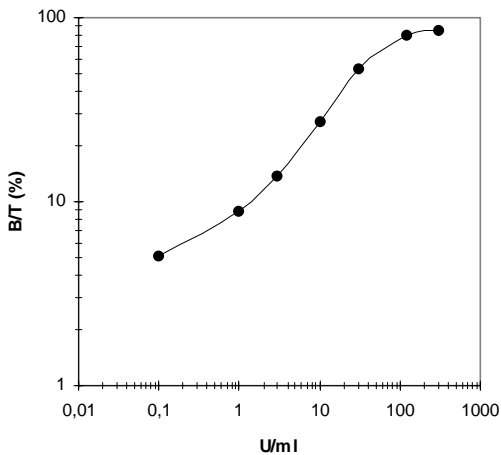
Do not use for evaluation!

Test tubes	cpm (a)	cpm (b)	cpm (mean)	$\frac{B}{T}$ %	U/ml
Total radioactivity T	29951	29878	29914	100 %	---
Calibrator 1	1580	1483	1532	5.1	0.1
Calibrator 2	2633	2692	2663	8.9	1
Calibrator 3	4125	4144	4134	13.8	3
Calibrator 4	8095	8989	8163	27.2	10
Calibrator 5	15624	16122	15872	53.1	30
Calibrator 6	24156	24054	24105	80.6	120
Calibrator 7 (optional)	25613	25217	25414	85.0	300
Control I	---	---	---	---	---
Control II	---	---	---	---	---
Patient 1	20117	20080	20099	67.2	53

Calculation of patient sample 1: $\frac{B}{T} (\%) = \frac{20099}{29914} \times 100 = 67 \%$

STANDARD CURVE

Typical example



REFERENCE VALUES

CentAK® anti-GAD ₆₅	
GAD ₆₅ antibodies negative	< 0.9 U/ml
GAD ₆₅ antibodies positive	≥ 0.9 U/ml

Normal range was established by Receiver Operating Characteristics (ROC) analysis.

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti-GAD₆₅ 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® anti-GAD₆₅ are arbitrary units.

CentAK® anti-GAD₆₅ passed the 1st to 3rd GADA Proficiency Study of the University of Florida, Gainesville, USA, in 1995 - 1997, and the 4th GADA Proficiency Study of the University of Louisiana, New Orleans, USA, in 1999, with 100 % specificity and 100 % sensitivity, each.

Parallelism of standards and serum samples

Dilutions of specimen in anti-GAD₆₅ free human serum are determined according to their expected theoretical values with CentAK® anti-GAD₆₅. 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 (¹²⁵I-GAD₆₅) does secure in direct assay principle of the test, that only anti-GAD₆₅ autoantibodies react and that any detectable cross reactions with autoantibodies to IA₂, 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-GAD₆₅, this value is found at approx. 0.6 U/ml.

Anti-GAD₆₅ 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-GAD₆₅ concentrations above approx. 0.6 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-GAD₆₅.

However, GAD₆₅ autoantibodies may be also present in a rare neurological disorder, Stiff-man Syndrome (SMS). Around 60 % of patients with SMS have GAD₆₅ autoantibodies in their serum. GAD₆₅ autoantibodies from patients with SMS have higher titers compared with those of patients with type 1 diabetes. That's why sera from patients with suspicion of SMS should be pre-dilute 1:50 and 1:100 with GAD₆₅ autoantibody negative sera. In patients with SMS GAD₆₅ autoantibodies occur also in cerebrospinal fluid.

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[®] anti-GAD₆₅




ASSAY SCHEME

1	Label test tubes*	CAL 1 - 6 (7)	CI - CII	Pat.-Sera 1, 2 etc.	T
2	Pipette Calibrators 1 - 6 (7) Control sera I - II Patient sera	20 µl	20 µl	20 µl	
3	Pipette Tracer (made from D and J)	50 µl	50 µl	50 µl	50 µl
4	Incubate**	2 hours (at room temperature)			
5	Pipette Protein A-Suspension (made from J and L)	50 µl	50 µl	50 µl	
6	Incubate**	1 hours (at room temperature)			
7	Pipette Buffer (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.