



Instructions for Use

Anti-21-Hydroxylase - Assay

Addison's Disease, Autoimmune Polyglandular Syndrome


125I-Radioassay for the Quantitative Determination of Antibodies against 21-Hydroxylase in Serum

CE

IVD

REF RA007/50

 50

 2 – 8 °C

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1. Principle of the Test

The assay kit provides reagents for the quantitative determination of autoantibodies against the 21-hydroxylase in serum. ¹²⁵Iodine labelled recombinant, human 21-hydroxylase is incubated with standards and patient sera and binds to the antibodies. The immune complexes are then precipitated with protein A. After centrifugation the radioactivity of the pellet is counted in a gamma counter. The amount of radioactivity is in proportion to the concentration of the antibodies. The quantification of unknown samples is achieved by comparing their activity with a reference curve prepared with known standards.

2. Precautions

- For in vitro diagnostic use only.
- Some reagents contain sodium azide as preservative. Avoid skin contact.
- This radioactive product assay only be received, stored as used by persons so authorized and by laboratories covered by such authorization. It must not be administered to humans or animals under any circumstances.
- Do not eat, drink or smoke where radioactive materials are being handled.
- Do not pipette by mouth.
- Wear disposable gloves when handling radioactive materials.
- The kit components "Negative and Positive Controls" are made with human serum. All sera used are tested for HIV I/II antibodies and HBsAg and found to be negative. However, because no test method can offer complete assurance that infectious agents are absent, these reagents should be handled as potentially biohazardous materials.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.

3. Storage and Stability

On arrival, store the kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents. Do not use components beyond the expiration date shown on the kit labels. Do not mix various lots of any kit component within an individual assay.

4. Contents of the Kit

- 4.1 **Tracer** **TRACER** 2 vials
125I- labelled 21-Hydroxylase
lyophilized, activity < 25 kBq per vial
- 4.2 **Protein A** **PROTEIN A** 1 vial
lyophilized
- 4.3 **Assay Buffer** **BUFFER** 1 bottle
120 ml, ready for use
- 4.4 **Standards** **CAL A – CAL F** 6 vials
each 0.15 ml, ready for use
Concentrations:
- | Standard | A | B | C | D | E | F |
|----------|---|---|---|----|-----|-------|
| U/ml | 0 | 1 | 5 | 50 | 500 | 5,000 |
- 4.5 **Control 1 & 2** **CON 1 – CON 2** 2 vials
each 0.15 ml, ready for use,
contains human serum
Concentrations see QC Certificate

Additional materials and equipment required but not provided:

- Pipettes for 20, 50 µl, and 1 ml
- Plastic tubes and suitable rack
- Centrifuge (preferable refrigerated) capable of at least 3,500 x g
- Suitable device for aspirating or decanting the tubes.
- Vortex mixer
- Gamma counter

5. Sample Collection and Storage

Serum should be used in the assay. Do not use lipemic or grossly haemolized specimen. Repeated freezing and thawing should be avoided. Samples which appear turbid should be centrifuged before assay to remove any particulate material.

Samples can be stored up to one week at 2 - 8 °C or at - 20 °C for longer periods.

6. Preparation of Samples and Reagents

6.1 Patient Samples

Allow the patient sera to reach room temperature, mix gently, and centrifuge, if necessary, to remove any particulate material.

6.2 Tracer **TRACER**

Reconstitute the lyophilized tracer with 1.3 ml Assay Buffer (4.3) per vial prior to use and mix carefully.

The reconstituted tracer should be used in the day of reconstitution. Stored at 2–8 °C the reconstituted tracer starts to deteriorate after a few days. Do not freeze the reconstituted tracer.

6.2 Protein A **PROTEIN A**

Reconstitute the lyophilized Protein A with 2.6 ml Assay Buffer (4.3) and mix carefully. Prior to use the Protein A has to be mixed thoroughly to ensure a uniform suspension.

Store at 2-8 °C after reconstitution and use within the shelf life of the kit. The suspension must not be frozen.

7. Test Procedure

- 7.1 Pipette each 20 μ l standards, controls and patient sera into the corresponding tubes.
- 7.2 Pipette each 50 μ l reconstituted tracer into all tubes.
- 7.3 Mix thoroughly (vortex) and incubate for 16 - 20 hours (over night) at 2-8 $^{\circ}$ C .
- 7.4 Mix the Protein A suspension thoroughly immediately prior to use and pipette each 50 μ l Protein A into all tubes (except Totals).
- 7.5 Mix thoroughly (vortex) and incubate for 1 hour at 2-8 $^{\circ}$ C.
- 7.6 Pipette each 1 ml **cold** (2-8 $^{\circ}$ C) Assay Buffer (4.3.) into all tubes (except T).
- 7.7 Mix thoroughly and centrifuge for 20 to 30 minutes at 3,500 x g, if possible in a refrigerated centrifuge.
- 7.8 Decant or aspirate the supernatant (except T).
- 7.9 Count all tubes for 1 minute in a gamma counter.

8. Calculation of Results

Construct a standard curve by plotting the mean cpm of each standard versus its corresponding concentration.

The concentration of the Controls and patient samples can then be read off the standard curve.

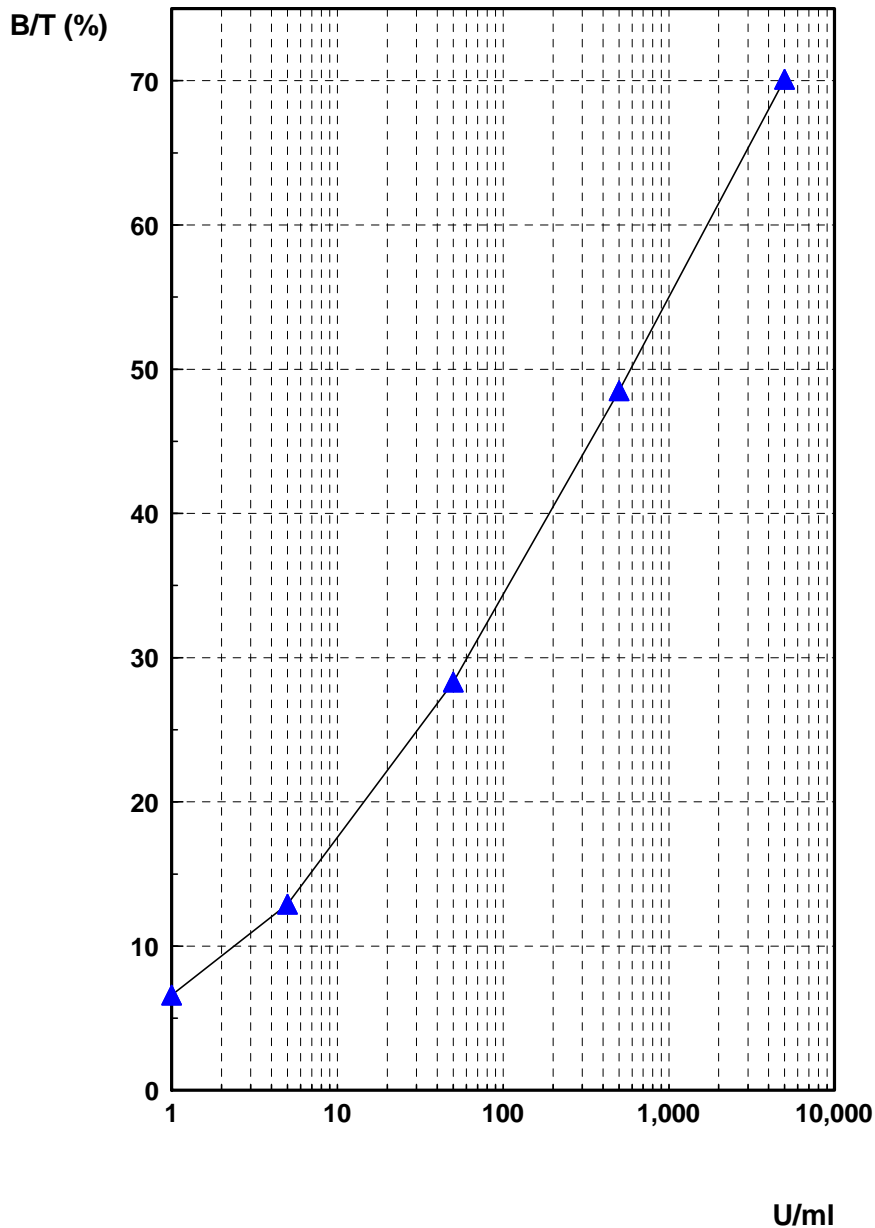
Alternatively, the cpm of the standards, controls, and patient samples can be related to the cpm of the Totals (B/T in %) and used on the y-axis for constructing the standard curve and for reading off the measured concentrations.

9. Typical Example

Typical results are shown in the following table

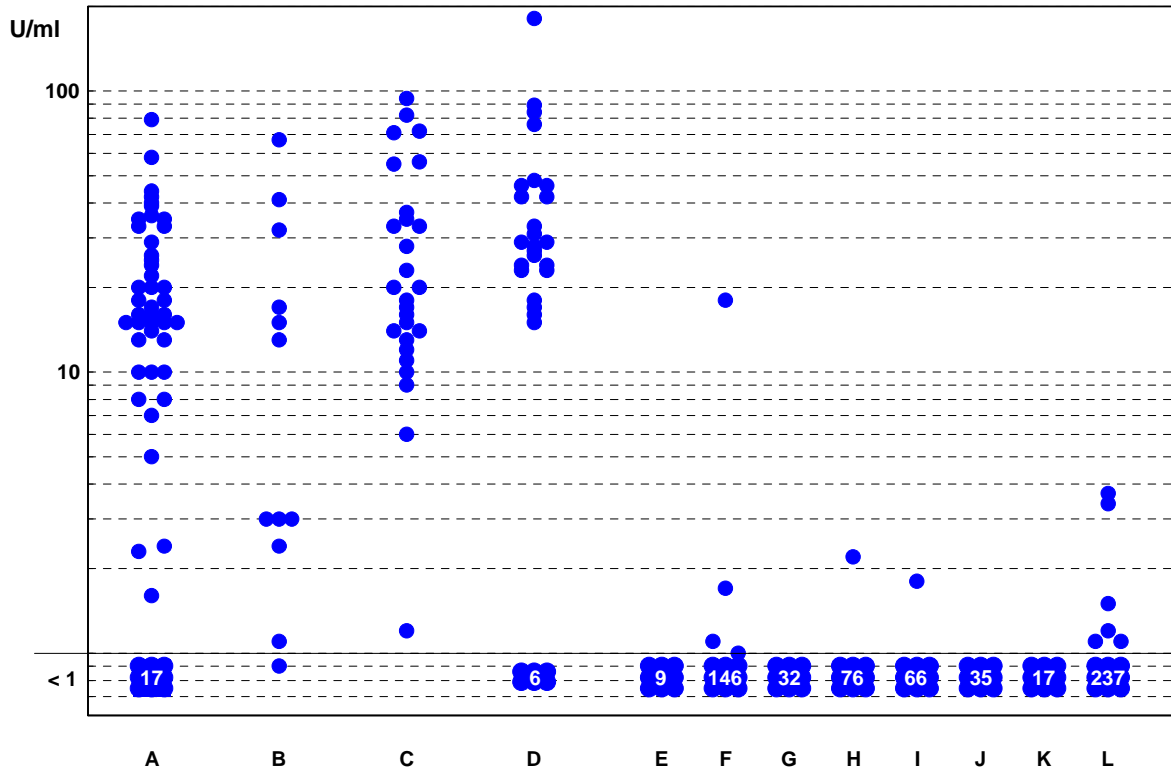
Concentrations (U/ml)	cpm1	cpm2	Mean	B/T (%)
Totals	32,847	32,968	32,908	
0	1,027	1,109	1,068	3.2
1	2,253	2,099	2,176	6.6
5	4,319	4,203	4,261	12.9
50	9,201	9,397	9,299	28.3
500	15,744	16,193	15,969	48.5
5,000	23,263	22,858	23,061	70.1

Typical Standard Curve



10. Reference Ranges

The normal range was determined by measuring the sera of 243 healthy blood donors. The mean value of this evaluation was 0.08 U/ml (standard deviation 0.36). This means that the upper limit of the normal range is around 1 U/ml. For establishing sensitivity and specificity samples from several patient groups were measured. The results are shown in the following figure.



Group A	Isolated Addison's Disease	N = 60
Group B	Polyglandular Autoimmune Syndrome Type I	N = 12
Group C	Polyglandular Autoimmune Syndrome Type II	N = 27
Group D	Immunofluorescence positive	N = 30
Group E	Addison's Disease due to Tuberculosis	N = 9
Group F	Insulin-Dependent Diabetes Mellitus (IDDM)	N = 150
Group G	Non-Insulin-Dependent Diabetes mellitus (NIDDM)	N = 32
Group H	Graves' Disease	N = 77
Group I	Hashimoto's Thyroiditis	N = 67
Group J	Myasthenia Gravis	N = 35
Group K	Premature Ovarian Failure	N = 17
Group L	Healthy Blood Donors	N = 243

From these measurements the following values result:

Reference Range < 1 U/ml

Sensitivity

Isolated Addison's (Group A) 72 %

APS Type I/II (Group B - C) 97 %

Specificity (Group E - L) 98 %

11. Precision

The following intra- and inter-assay coefficient of variation were measured:

intra-assay precision (n=20)		
sample	mean (U/ml)	CV (%)
3	17.4	5.1
4	5.7	5.6

inter-assay precision (n=25)		
sample	mean (U/ml)	CV (%)
1	7.5	6.6
2	22.4	5.9

12. Literature

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- Further literature available upon request

Pipetting Scheme

		T	B ₀	Standards	Controls	Patients
Standard A	μl		20			
Standard B-F	μl			20		
Control 1 & 2	μl				20	
Patient sample	μl					20

¹²⁵ I-Tracer	μl	50	50	50	50	50
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Mix thoroughly and incubate for 16 - 20 hours (overnight) at 2 - 8 °C

Protein A	μl		50	50	50	50
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Mix thoroughly and incubate for 1 hour at 2 - 8 °C

Assay Buffer	ml		1	1	1	1
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Centrifuge (2-8 °C) for 20 to 30 minutes at min. 3, 500 x g

Decant or aspirate the supernatant

Measure for 1 minute in a gamma counter