

## Organ-Specific Autoimmunity

- Thyroid Disease
- Diabetes Mellitus
- Addison's Disease
- Myasthenia Gravis

# Autoimmune Thyroid Diseases (ATD)

Autoantibodies in ATD are directed to the thyroid specific proteins which are Thyroglobuline (Tg), Thyroid peroxydase (TPO) and Thyroid Stimulating Hormone Receptor (TSH-R).

Autoimmune responses to these antigens vary in different forms of ATD:

- In Graves' disease, autoantibodies are directed mainly to TSH-R.
- Hashimoto's thyroiditis is characterised by autoantibodies directed to Tg and TPO and in some cases to TSH-R.
- Postpartum thyroid dysfunction is an organ specific autoimmune disorder associated with Tg and TPO autoantibodies.

Patients with autoimmune thyroid diseases are at heightened risk of developing a second autoimmune problem: Addison's disease, type 1 diabetes, pernicious anaemia, myasthenia gravis.

# TR-AB-CT

## TSH RECEPTOR AUTOANTIBODIES

### Clinical interest

- Differential diagnosis of Grave's disease
- Prediction of the outcome of Grave's disease after treatment
- Prediction of foetal / neonatal hyperthyroidism
- Risk factor for Grave's orbitopathy

### Principle of the assay

#### Radioreceptor assay in coated tube

- TSH-R antibodies in patient sera interact with TSH receptors coated onto tubes
- Bound TSH-R antibodies inhibit the binding of labelled TSH to the receptors
- Results are expressed in U/L (NIBSC 90/672) or in inhibition of TSH binding index

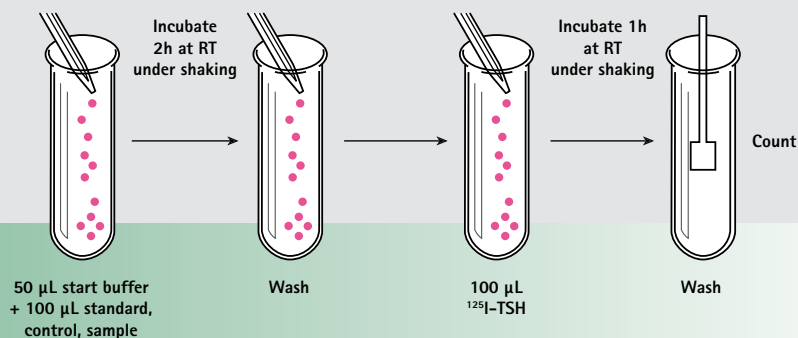
### Characteristics

- Standard range: 0-40 U/L
- Detection limit: 0.3 U/L
- Functional sensitivity: 0.8 U/L
- Sample: serum
- Shelf life: 6 weeks

### Expected values

U/L	≤ 1	1.1 - 1.5	> 1.5
TSH-R-Ab	Negative	Grey zone	Positive

### ASSAY PROCEDURE



# TPO-AB-CT

## THYROID PEROXYDASE AUTOANTIBODIES

### Clinical interest

- Prediction of postpartum thyroiditis in at risk women
- Diagnosis, follow-up, prognosis of thyroiditis in Hashimoto's thyroiditis
- In subclinical hypothyroidism, risk factor of developing overt thyroid disease

### Principle of the assay

#### Competition assay in coated tube

- Competition between TPO-Ab in samples and monoclonal TPO-Ab on the coated tube for a limited quantity of  $^{125}\text{I}$  labelled TPO antigen
- Results are expressed in U/ml

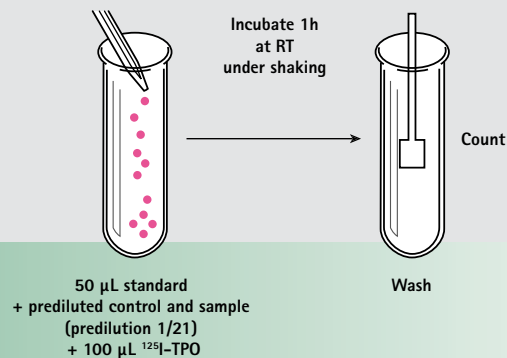
### Characteristics

- Standard range: 0-10000 U/mL
- Detection limit: 8 U/mL
- Sample: serum, plasma
- Shelf life: 10 weeks

### Expected values

U/mL	< 70	70 - 130	> 130
TPO-Ab	Negative	Grey zone	Positive

### ASSAY PROCEDURE



# TGAB I STEP

## THYROGLOBULIN AUTOANTIBODIES

### Clinical interest

- Differentiated thyroid carcinoma follow-up

### Principle of the assay

#### Competition assay in coated tube

- Competition between Tg-Ab in samples and monoclonal Tg-Ab on the coated tube for a limited quantity of  $^{125}\text{I}$  labelled Tg antigen
- Results are expressed in IU/mL

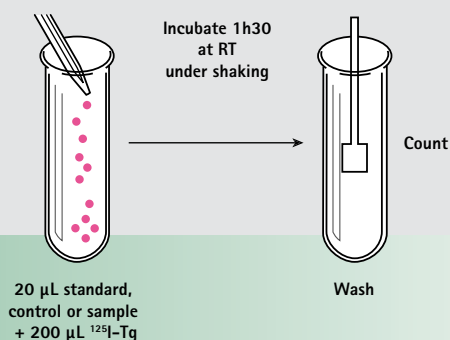
### Characteristics

- Standard range: 0-2000 IU/mL
- Detection limit: 6 IU/mL
- Functional sensitivity: 15 IU/mL
- Sample: serum
- Shelf life: 6 weeks

### Expected values

IU/mL	< 30	30 - 70	> 70
Tg-Ab	Negative	Grey zone	Positive

### ASSAY PROCEDURE



# Diabetes Mellitus (D.M.)

Type 1 diabetes mellitus (T1DM) is the consequence of the destruction of  $\beta$  cells of the Langerhans islets, eventually leading to absolute insulin deficiency in most cases.

The majority of the islets undergoes a destructive process controlled by an immune mediation.

Interaction between genetic and environmental factors induces secretion of diabetes autoantibodies:

- Glutamic Acid Decarboxylase Autoantibodies: GAD-AB
- Tyrosine Phosphatase Autoantibodies: IA2-AB
- Insulin Autoantibodies: IAA

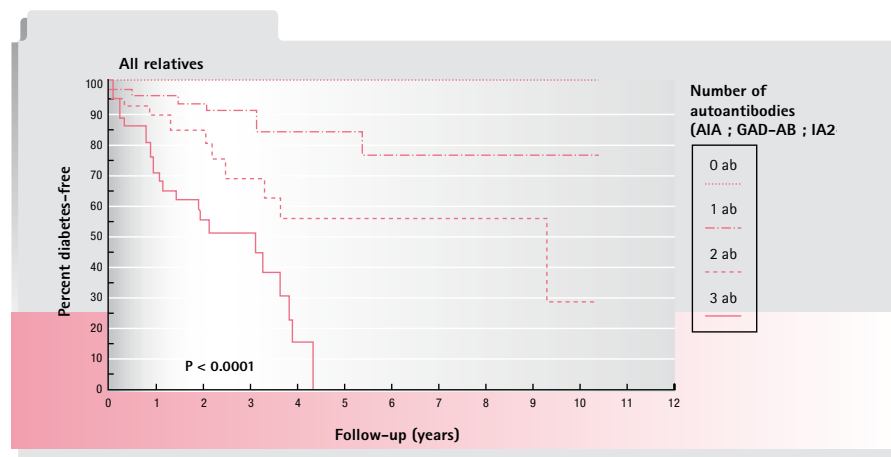
These antibodies can be used as:

- diagnostic markers to help to define the aetiology and to classify the disease origin (Immune or not)
- monitoring markers
- prognosis factor.

They are potentially valuable to predict the disease through population screening :

"We conclude that the presence of two or more autoantibodies (out of IAAs, GAAs and ICA512bdcAAs) is highly predictive of the development of type 1 diabetes among relatives"<sup>(1)</sup>

(1) "Prediction of Type 1 Diabetes in First-Degree Relatives Using a Combination of Insulin, GAD and ICA512bdc/IA-2 Autoantibodies". *Diabetes*, 1996, 45 : 926-933  
Charles F. Verge, Roberto Gianini, Eiji Kawasaki, Liping Yu, Massimo Pietropaolo, Richard A. Jackson, H. Peter Chase and George S. Eisenbarth.



# GAD-AB

## GLUTAMIC ACID DECARBOXYLASE AUTOANTIBODIES

### Clinical interest

- Prediction and prevention of type 1 diabetes
- Severity of the clinical state
- Classification of autoimmune diabetes
- Screening of population: identification of high-risk individuals
- Identification of the latent autoimmune diabetes in adults
- Type 2 diabetes associated with neuropathy
- In the 2005 DASP study, the GAD-AB kit showed 95% specificity and 84% sensitivity

### Principle of the assay

#### Radioimmunoassay

- GAD autoantibodies in sera interact with iodine labelled recombinant GAD
- A solid phase protein A is added to precipitate the complexes previously formed
- Results are expressed in U/mL (1 U/mL is equivalent to 25 U/mL of NIBSC 97/550)

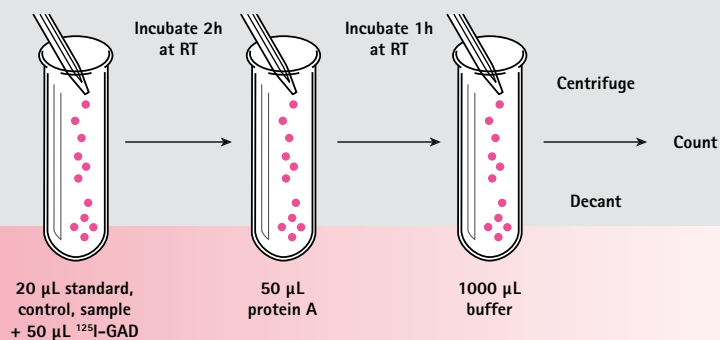
### Characteristics

- Standard range: 0-300 U/mL
- Detection limit: 0.11 U/mL
- Sample: serum, EDTA plasma
- Shelf life: 6 weeks

### Expected values

U/mL	≤ 1	> 1
GAD-Ab	Negative	Positive

### ASSAY PROCEDURE



# IA2-AB

## ANTI-TYROSINE PHOSPHATASE AUTOANTIBODIES

### Clinical interest

- Prediction of the progression to clinical symptoms
- Severity of the clinical state
- Screening of population: identification of high-risk individuals
- In the 2005 DASP study, the IA2-AB kit showed 100% specificity and 70% sensitivity

### Principle of the assay

#### Radioimmunoassay

- IA2 autoantibodies in sera interact with iodine labelled recombinant IA2
- A solid phase protein A is added to precipitate the complexes previously formed
- Results are expressed in U/mL (1 U/mL is equivalent to 125 U/mL of NIBSC 97/550)

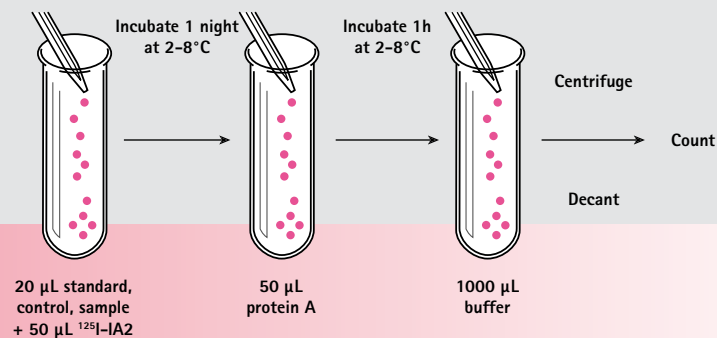
### Characteristics

- Standard range: 0-50 U/mL
- Detection limit: 0.19 U/mL
- Sample: serum, EDTA plasma
- Shelf life: 6 weeks

### Expected values

U/mL	≤ 1	> 1
IA2-Ab	Negative	Positive

### ASSAY PROCEDURE



## AAI

INSULIN  
AUTOANTIBODIES

## Clinical interest

- Early sign of an ongoing autoimmune process
- Discriminant analysis between insulin autoantibodies and insulin antibodies induced by the therapy

## Principle of the assay

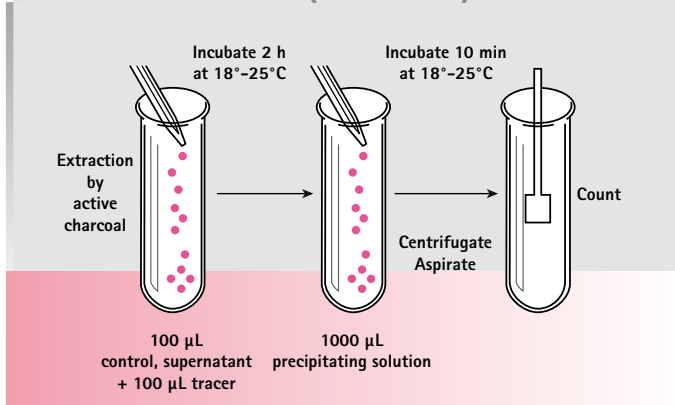
## Semi quantitative assay

- **Total anti insulin antibodies (total AIA):**  
dissociation of the immune complexes, insulin adsorption by charcoal then radioimmuno-precipitation
- **Free anti insulin antibodies (free AIA):**  
Radioimmunoprecipitation:  
IAA in sera interact with  $^{125}\text{I}$  labelled insulin  
PEG is added to precipitate the complexes previously formed  
Results are expressed in binding percentage

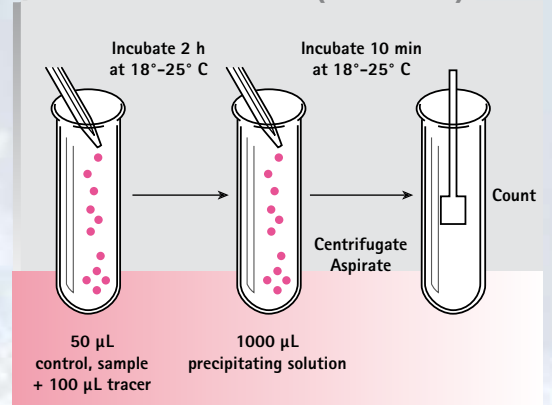
## Expected values

- Normal values < 5.5 % B/T

## ASSAY PROCEDURE (TOTAL AIA)



## ASSAY PROCEDURE (FREE AIA)



# Addison's Disease

## (or autoimmune adrenal disease)

Autoimmune destruction of the adrenal cortex is the most common cause of Addison's disease.

Steroid 21-hydroxylase (21-OH) is a major adrenal autoantigen and 21-OH autoantibodies are important markers of autoimmune adrenal disease. This is the case whether the disease presents as isolated Addison's disease or as part of the autoimmune polyglandular syndromes (APS) type I or type II.

# 21-OH-AB

## STEROID 21-HYDROXYLASE AUTOANTIBODIES

### Clinical interest

- Assessment of adrenal autoimmunity
- Prediction of adrenal insufficiency (identification of the subjects at high risk)
- Early detection of adrenal insufficiency
- Aid in therapy (early replacement therapy)

### Principle of the assay

#### Radioimmunoassay

- 21-OH antibodies in patient sera, calibrators and controls interact with highly purified recombinant  $^{125}\text{I}$  21-OH
- After an overnight incubation, solid phase protein A is added to precipitate the complexes previously formed
- Results are expressed in U/mL

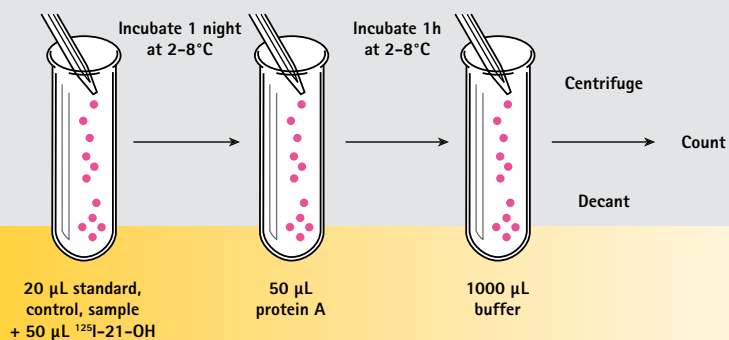
#### Characteristics

- Standard range: 0-5000 U/mL
- Detection limit: 0.16 U/mL
- Sample: Serum
- Shelf-life: 6 weeks

### Expected values

U/mL	Positive
21-OH Ab	$\geq 1.0$ U/mL

### ASSAY PROCEDURE

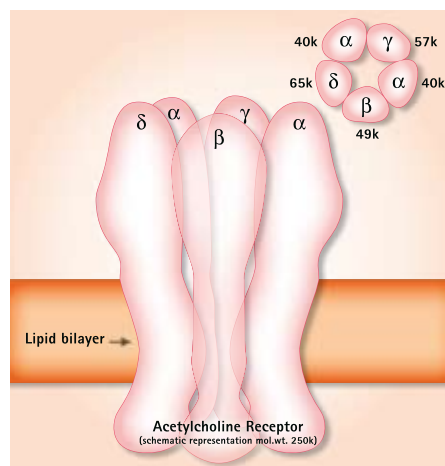
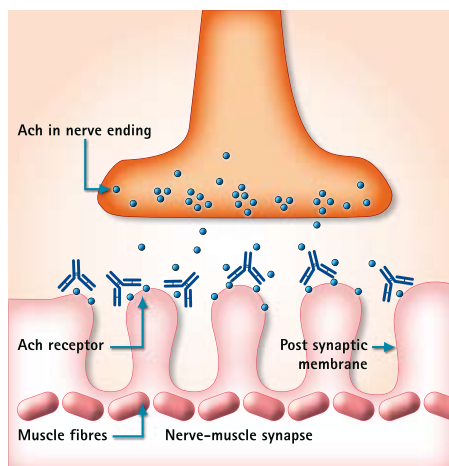


# Myasthenia Gravis (M.G.)

Myasthenia Gravis (M.G.) is a disorder of neuromuscular transmission with muscle weakness and fatigue on exertion. It results from a blockade of the neuromuscular junction that is caused by antibodies directed against the nicotinic acetylcholine receptor. The acetylcholine receptor has been cloned and its structure determined. It is formed by five subunits ( $2\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ). Binding of acetylcholine causes opening of the cation channel which allows  $\text{Na}^+$  to enter the molecule. All subunits are required for a fully functional receptor.

In M.G., the function of the nerve-muscle synapse is affected by the autoimmune process. It is postulated that the major mechanism responsible for the dysfunction is related to the increase of the receptor internalisation and degradation rather than to direct blocking of binding sites.

Adult and foetal forms of the acetylcholine receptor differ by one of their subunit (the  $\gamma$  subunit in foetal receptor is replaced by the  $\epsilon$  subunit in adult receptor). In some sera, AchR-AB recognise the foetal form preferentially whereas in other sera, AchR-AB recognise the adult form preferentially.



# ACHR-AB

## ACETYLCHOLINE RECEPTOR AUTOANTIBODIES

### Clinical interest

- Diagnostic of Myasthenia Gravis
- Follow-up of the disease progression

### Principle of the assay

#### Radioimmunoassay

- Detergent solubilized acetylcholine receptors labelled with  $^{125}\text{I}$ -a bungaratoxin are incubated with test sera and the resulting complex is immunoprecipitated with anti human IgG
- A balanced mixture of solubilized foetal and adult forms of the receptor represents the optimum preparation and is used to maximize the assay sensitivity

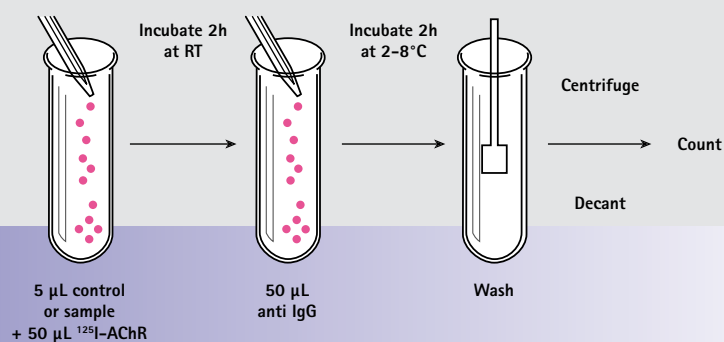
### Characteristics

- Detection limit: 0.02 nmol/L
- Sample: serum, EDTA plasma
- Shelf life: 6 weeks

### Expected values

nmol/L	$\leq 0.2$	0.2 - 0.5	$> 0.5$
ACHR-Ab	Negative	Grey zone	Positive

### ASSAY PROCEDURE



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