

ANTITHROMBIN III RID

Determination of the Antithrombin III protein, by radial immunodiffusion plate

TEST SUMMARY

The examined protein, diffusing in agarose gel containing a specific antibody will form an immuno-complex, visible as a ring around the well. The ring diameter is direct proportional to the concentration of the analysed protein. The proportion corresponds to the diffusion time. In fact, at the end (72h), the square of diameter will be in linear proportion to the concentration of the sample.

With the plate is supplied a reference table in which each diameter of the halo is associated a concentration.

SAMPLES

Serum, plasma. Stability 6 days at 4°C.

REAGENTS

Plate: Agarose gel containing the goat antiserum Antithrombin III.

REAGENTS PREPARATION AND STORAGE

The plates are ready to use.

The reagents are stable until expiration date on the label if preserved horizontal at 2-8°C.

Stability after opening: two weeks if, after the first use, is preserved well closed at 2-8°C.

The plate can be used for further 2 weeks checking the accuracy by a control serum.

MATERIALS REQUIRED BUT NOT SUPPLIED

Micropipette to 5 µl, slide rule, lens of measure, current laboratory instrumentation.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.

Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

PROCEDURE

Remove the plate from its envelope and leave to stand at room temperature for few minutes so that any condensed water in the wells can evaporate. Fill the wells with 5 µl of sample and/or controls and wait it has been completely adsorbing before handling the plate. Close the plate and place it in a moist chamber for 72 hours.

RESULTS INTERPRETATION

Measure the precipitating ring with an appropriate ruler or measuring lens however a system which provides a maximum error of 0.1 mm. Read on enclosed reference table the concentration value corresponding to the precipitating ring diameter.

The control serum, to be used always, should give a ring which differs by a maximum of 0.2 mm from the value reported in the table.

Reading 18 hours (kinetic method)

You can read the results after 18 hours of the sample deposition, although the growth of the zones is not yet complete. In this case it is necessary to deposit at least 3 controls with different values.

Curve that plots the square of the precipitating ring and the logarithm of the concentrations of the controls. You should get a interpolating curve that can be approximated to a straight line only for low values while for higher values may be bent slightly. The values of the samples are determined by interpolation.

NOTES

- The diffusion time and the reading time depend on the concentration and the specific diffusion protein. After 72 h the diffusion of the protein at any concentration is completed. For lower concentration it is possible to read in lower times (i.e. 36 h), however in such cases it is advisable to read again after 3/5 hours. If the diameter is still the same it is possible to set the concentration, on the contrary, if the diameter is different, ring should be remeasured after a further 3/5 hours.
- The reference table attached is valid only for the specific lot of the plate. Do not use with different lot.

CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following human based control sera are available:

IC00700 1 x 1 ml
(single for Antithrombin III)

TEST PERFORMANCE

Precision

Intra-assay (n = 10)	mean	SD (mg/dl)	CV %
sample 1	35.61	0.54	1.52
sample 2	67.8	0.63	0.93

Inter-assay (n = 20)	mean	SD (mg/dl)	CV %
sample 1	35.5	0.44	1.26
sample 2	67.95	0.68	1.01

Methods comparison

A comparison between LTA and a commercially available product gave the following results on 70 samples:

Antitrombin III LTA = x
Antitrombin III competitor = y
n = 70

$$y = 0,98x + 0,77 \quad r = 0,9992$$

Measure's limit

7 – 80 mg/dl

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

EXPECTED VALUES

Serum 17 – 30 mg/dl
Plasma 22 – 39 mg/dl

Clinical relevance

The Antithrombin III present in the blood is a glycoprotein produced by the liver which acts as a cofactor of the heparin, plays an action anticoagulant.

Low concentrations of antithrombin III may indicate increased risk of phlebitis, pulmonary embolism, deep vein thrombosis, etc. ..., and can be observed in the case of liver failure, liver cirrhosis, and nephrotic syndrome.

As with any diagnostic procedures if the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.

PACKAGING

CODE **RK00350**
Antithrombin III 1 x 15 wells

REFERENCES

Mancini & coll.-Immunochemistry. 2:235 (1965)
Fahey & coll.- J. Immunol. 94 : 84 (1965)
Pasquinnelli, Diagnostica e Tecniche di laboratorio Vol. 1, p.703.

MANUFACTURER

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SYMBOLS

- IVD Only for IVD use
- LOT Lot of manufacturing
- REF Code number
- Storage temperature interval
- Expiration date
- Warning, read enclosed documents
- Read the directions
- Biological risk

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