C3 RID

Determination of the C_3 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 C_3.

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 is available on request the following human serum titred suitable for use as a calibrator or control:

IC00200 Serumprotein Calibrator 7 Parameters (for α-1 acid Glycoprotein, C3, C4, IgA, IgG, IgM and Transferrin)

TEST PERFORMANCE

Precision

Intra-assay (n = 10)	mean	SD (mg/dl)	CV %
sample 1	102.36	1.81	1.77
sample 2	183.40	2.54	1.39

Inter-assay (n = 20)	mean	SD (mg/dl)	CV %
sample 1	101.51	1.95	1.92
sample 2	184.21	1.98	1.07

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

C_3 LTA = x
C_3 competitor = y
n = 70

y = 1,008x + 0,366 r = 0,9997

Measure's limit
26 – 300 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

C_3 91 – 156 mg/dl

Clinical relevance
The C3 fraction is a protein of the complement, synthesized by the liver, which is activated in the presence of bacterial cells, or immune complexes. Following activation of complement there is the immune response by the organism.
An increase of the values of C3 fraction may be due to inflammation, autoimmune diseases, chronic infections, myocardial infarction
Decreased values of C3 fraction may be due to a variety inflammatory and infectious diseases, kidney disease, liver disease, autoimmune hemolytic anemia
The C3 fraction of complement, together with the C4 fraction, is the most frequently measured.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	RK00400
C_3	1 x 15 wells

REFERENCES

Mancini & coll.-Immunochemistry. 2:235 (1965)
Fahey & coll.- J. Immunol. 94 : 84 (1965)

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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