

Determination of the C₄ 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 (procedure 1-3), while after shorter period of diffusion the square of diameter will be in a logarithmic proportion to the concentration (procedure 2). In both cases, a calibration curve should be constructed, using at least three calibration points. However a reference table is provided showing the relation between any concentration and the end of the procedure.

SAMPLES

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

REAGENTS

Plate: Agarose gel containing the goat antiserum C₄.

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, 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. Wait the required incubation period (72 hours for the procedure 1 or 3, 18 hours for the procedure 2). To quicken analysis time it is possible to put the plates in a thermostat.

RESULTS INTERPRETATION

Measure the precipitating ring to the nearest 0.1 mm, after the required period according to the procedure should be followed, 72 or 18 hours.

Procedure 1

Construct a curve that plots the square of the precipitating ring versus the concentrations of the controls. A straight line, with an intercept in the range 10-11 mm, should be obtained. Sample values are determined by interpolation.

Procedure 2

Construct a curve that plots the square of the precipitating ring versus the logarithm concentrations of the controls. The graph will be a straight line only for low values. Sample values are determined by interpolation.

Procedure 3

Read on enclosed reference table the concentration value corresponding to the precipitating ring diameter. The ring value is obtained from the control serum (that has to be used every time) which should have a confidence limit of 0.2 mm (from the reference table).

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.

CALIBRATION

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

IC00400 Multiset multiparametric 4 x 1 ml
(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	27.84	1.27	4.57
Sample 2	53.72	0.67	1.26

Inter-assay (n = 20)	mean	SD (mg/dl)	CV %
Sample 1	27.72	1.12	4.07
Sample 2	53.4	0.79	1.49

Methods comparison

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

C₄ LTA = x
C₄ competitor = y
n = 70

$$y = 0,967x + 1,43$$

$$r = 0,9681$$

Measure's limit

7 – 90 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

C₄ 20 – 50 mg/dl

PACKAGING

CODE **RK00500**
C₄ 1 x 15 wells









REFERENCES

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

MANUFACTURER

Meridian Healthcare srl
Tel. +39 095 725 68 69 Fax: +39 095 725 44 54
info@meridianhealthcare.it
www.meridianhealthcare.it 

SYMBOLS

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

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