COMPLEMENT C4 (C4)

IMMUNOTURBIDIMETRIC ASSAY FOR SERUM COMPLEMENT C4
RX SERIES

INTENDED USE

A Complement C4 test system is a device intended for the quantitative *in vitro* determination of Complement C4 concentration in serum. This product is suitable for use on RX **series** instruments which includes the RX **daytona** and the RX **imola**.

Cat. No.			
CM 3846	R1.	Assay Buffer	3 x 20 ml
	R2.	Antibody Reagent	3 x 6 ml

GTIN: 05055273201628

CLINICAL SIGNIFICANCE[1]

Complement is a complex biological system, which works in conjunction with antibody and other factors to protect the body from invasion by pathogens. When activated by either the classical or alternative pathway, complement acts on biological membranes and may cause cell death. The human complement cascade consists of several distinct plasma proteins.
Complement C4 levels are important in determining inherited or acquired deficiencies. Conversely, levels may rise in a variety of inflammatory and necrotic disorders as part of the acute-phase plasma protein response.

PRINCIPLE

Sample is reacted with a buffer containing antibody specific for human complement C4 (ϑ_1E-globulin). The absorbance (340 nm) of the resulting turbid solution is proportional to the concentration of C4 in the sample. By constructing a standard curve from the absorbance of standards, C4 concentration of sample can be determined.

SAMPLE COLLECTION AND PREPARATION

Serum should be stored at between +2 to +8°C for a maximum of 1 week or stored frozen at –20°C for a maximum of 6 months (do not refreeze).

REAGENT COMPOSITION

Contents	Initial Concentration of Solutions
R1. Assay Buffer	
Polyethylene Glycol	maximum 6% (w/v)
Tris/HCl buffer	20 mmol/l, pH 7.4
Sodium Chloride	150 mmol/l
Sodium Azide	0.1% w/v
R2. Antibody Reagent	
Anti (human) Complement C4	
Tris/HCl buffer	20 mmol/l, pH 7.4
Sodium Chloride	150 mmol/l
Sodium Azide 0.09% w/v	

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Solutions R1 and R2 contain Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water for 10 minutes. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents, flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Health and Safety Data Sheets are available on request.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

R1. Assay Buffer
Supplied ready for use. Stable up to expiry date when stored at +2 to +8°C.

R2. Antibody Reagent
Supplied ready for use. Stable up to expiry date when stored at +2 to +8°C.

MATERIALS PROVIDED

Assay Buffer
Antibody Reagent

MATERIALS REQUIRED BUT NOT SUPPLIED

Randox Liquid Assayed Specific Protein Calibrator, Cat. No. IT 2691
Randox Liquid Assayed Specific Protein Controls:

Level 1	Cat. No. PS 2682
Level 2	Cat. No. PS 2683
Level 3	Cat. No. PS 2684

RX **series** Saline, Cat. No. SA 3854

PROCEDURE NOTES

Place calibration series on the analyser in the order lowest to highest. Enter lot specific values based on those given in Specific Protein Calibrator insert.

The Chemistry parameters for Randox Dedicated RX **series** Assays are predefined on the hard drive of the analyser PC. The required programs should be downloaded to the analyser software. Please note that the predefined chemistry parameters use SI units. If alternative units are required, these can be edited by the user. In this case, the technical range should be edited in accordance with the users selected units. All necessary instructions are encoded on the bar code. If the barcode cannot be read by the analyser, enter manually the series of numbers given beneath the barcode. If problems continue, contact Randox Laboratories RX Support, Northern Ireland +44 (0) 28 9445 1070.

CALIBRATION
0.9% NaCl as zero calibrator and Randox Liquid Assayed Specific Protein Calibrator are recommended for calibration.

QUALITY CONTROL
Randox Liquid Assayed Specific Protein Controls - Levels 1, 2 and 3 are recommended for Quality Control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Technical Services, Northern Ireland + 44 (0) 28 9445 1070.

Quality control requirements should be determined in conformance with government regulations or accreditation requirements.

SPECIFICITY/INTERFERENCE
Serum analytes other than C4 were added to serum spiked with C4. The following analytes were tested up to the following levels and found not to interfere:

Haemoglobin	1000 mg/dl
Free Bilirubin	25 mg/dl
Conjugate Bilirubin	25 mg/dl
Triglycerides	250 mg/dl
Intralipid	200 mg/dl

Physiological changes in serum or plasma analyte concentrations can be caused by a number of substances. Comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. The listed reference contains specific details on known potential interfering substances [2]. The user must remain vigilant to the possible effect on results of unknown interferences from medications or endogenous substances. All patient results must be evaluated in light of the total clinical status of the patient.

EXPECTED VALUES[3,4]

Healthy adults:
20 Years : 15 - 43 mg/dl
30 Years : 16 - 46 mg/dl
40 - 70 Years : 18 - 49 mg/dl

Newborns:
7 - 23.5 mg/dl

Children:
3 Months : 9 - 30.5 mg/dl
6 Months : 10 - 35 mg/dl
9 Months : 11.5 - 39 mg/dl
12 Months : 12 - 40 mg/dl
2 - 10 Years : 12.5 - 42.5 mg/dl
12 - 18 Years : 14 - 43 mg/dl

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographic location of the population.

CONVERSION FACTORS
mg/dl x 0.01 = g/l

SPECIFIC PERFORMANCE CHARACTERISTICS
The following performance data was obtained using a RX **daytona** analyser at 37°C.

ASSAY RANGE
The range of this assay is approximately 3.41 - 152 mg/dl depending on the lot-specific value of the calibrator used. In the event of a rerun, this is extended to approximately 1520 mg/dl.

This is dependent on the lot specific value of the calibrator in use.

SENSITIVITY
The minimum detectable concentration of C4 was determined as 3.41 mg/dl.

PROZONE
Antigen excess effects are not noted until levels approach 663 mg/dl.

PRECISION

Intra Assay

	Level 1	Level 2	Level 3
Mean (mg/dl)	13.2	43.0	90.8
SD	0.513	0.638	4.41
CV(%)	3.88	1.48	4.85
n	20	20	20

Inter Assay

	Level 1	Level 2	Level 3
Mean (mg/dl)	13.6	43.1	88.5
SD	0.624	1.34	4.31
CV(%)	4.61	3.11	4.87
n	20	20	20

CORRELATION
This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:

Y = 0.99 X - 0.06
and a correlation coefficient of r = 0.98

40 patient samples were analyzed spanning the range 24 to 66 mg/dl.

REFERENCES
1. Alper, C.A. and Rosen, F.S. (1975). Adv. Intern. Med. **20:** 61-88.
2. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Washington, DC: AACC Press; 2000.
3. Johnson, A.M. (1996) Complement Component C4. In "Richie, R.F., Novolotskaia, O. eds. Serum proteins in Clinical Medicine", Scarborough: Foundation for Blood Research: 10.02-1-11.
4. Labor, T. (1998). In "Clinical Laboratory Diagnostics. Use and Assessment of Clinical Laboratory Results". 1st edition p. 696.

The presence of a vertical bar in the margin indicates a technical update from the previous revision.

EC REP Randox Teoranta, Meenmore, Dungloe, Donegal, F94 TV06, Ireland

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